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NIOSH GRANTS

RESEARCH and DEMONSTRATION PROJECTS

ANNUAL REPORT
FISCAL YEAR 1987

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

ORIGINAL

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NIOSH

RESEARCH AND DEMONSTRATION GRANTS

FISCAL YEAR 1987

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health
Atlanta, Georgia 30333

May 1988

I

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DHHS (NIOSH) Publication No. 88-115

FOREWORD

Under the provisions of the Occupational Safety and Health Act of 1970 and the Federal Mine Safety and Health Amendments Act of 1977, the National Institute for Occupational Safety and Health (NIOSH) is required to develop recommendations for standards to protect American workers against diseases and injuries resulting from exposure to occupational risks. In doing this, NIOSH also carries out a comprehensive program of research, service, training, and related activities. In addition to a substantial scientific agenda in intramural research, NIOSH supports a limited amount of excellent extramural research in occupational safety and health, as an occupational element of its research program.

In my second term as Director of NIOSH, I am especially interested in encouraging the implementation of the 10 "Proposed National Strategies for Prevention of Leading Work-Related Diseases and Injuries," developed during my first term. To turn these broadly accepted principles into effective preventative action, will require bold vision and serious commitment by a wide coalition of organizations and professional disciplines. In NIOSH itself we are significantly refocussing our resources so that key needs depicted in the *Strategies* are addressed by our research program including the extramural research element. A listing of the areas of particular interest to us is given in the *Program Announcement* included in this report.

Because the strength of the NIOSH extramural research program lies in the informed creativity of the American scientific community, we publish this report as a means of disseminating information on what needs doing. We seek to stimulate submission of imaginative proposals for research of high relevance to prevention of the leading occupational diseases and injuries, as well as of high scientific quality.

We invite the interest of investigators in the biomedical sciences, engineering, and related disciplines. By including descriptions of active grants during fiscal year 1987 (October 1, 1986 to September 30, 1987), we hope to provide a readily available source of information on the nature and scope of the research grants program of NIOSH. If you have further questions about these activities, please contact Dr. Roy M. Fleming, NIOSH Grants Office, Building 1, Room 3053, Atlanta, GA 30333.

J. Donald Millar, M.D., D.T.P.H. (Lond.)
Assistant Surgeon General
Director, National Institute for
Occupational Safety and Health
Centers for Disease Control

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IV

TABLE OF CONTENTS

Page

FOREWORD iii

INTRODUCTION 1

PROGRAM ANNOUNCEMENT 2

GLOSSARY 9

RESEARCH AND DEMONSTRATION GRANT SUMMARIES BY PROGRAM AREA 10

Occupational Lung Diseases

Research Project Grants (R01)
- (00923) Aerosol Deposition in the Human Respiratory System 10
- (01035) Health Risks in the Vermont Granite Industry 13
- (01970) Experimental Pneumoconiosis of Sandblasting Substitutes 15
- (02029) Respiratory Hazards of Poultry Workers 17
- (02076) Pulmonary Toxicity of the Semiconductor, Gallium Arsenide 19
- (02114) Epithelial Surface Proteins: Markers of Cancer Risk 21
- (02116) Tannin as a Byssinotic Agent-Effect on Endothelial Cells 23
- (02185) Polonium-210 Radiation and Dithiols 25
- (02264) Mechanism and Treatment of Phosgene Poisoning 27
- (02332) Effect of Particle Load on Alveolar Clearance 28

Career Development Grants (K01)
- (00002) Lung Collagen in Silicosis Fibrosis Mechanisms 29
- (00007) Oxygen Free Radicals in Pulmonary Agents 31
- (00018) Silicosis: Immunological Abnormalities 32
- (00019) Effect of Mineral Exposure on Macrophage Function 34
- (00022) Gas and Particle Deposition in Tracheobronchial Airways 36
- (00065) Macrophage, Particulate, and Carcinogen Metabolism 38
- (00067) Inhaled Toxic Agents: An Evaluation of Dose 39

Small Grants (R03)
- (02098) Occupational Health Factors in Farmer's Lung Disease 40
- (02189) Coal Workers' Respiratory Disease Program Evaluation 42
- (02265) Cotton Extract and Human Airway Hyperresponsiveness 44
- (02425) Immune Responsiveness in Chlorine Exposed Rats 46

Conference Grants (R13)
- (02117) International Conference on Biological Mechanisms 47

Musculoskeletal Injuries

Research Project Grants (R01)
- (01929) Occupational Lifting and Low Back Muscular Fatigue 48
- (02084) Back Pain in Industry: A Prospective Epidemiologic Study 49

Small Grants (R03)
- (02178) Wrist Orientation Effect on Grip Strength and Endurance 51
- (02229) Maximum Acceptable Lifts in a Sitting Position 52
- (02287) Mixture Model for Low Back Pain Rehabilitation 54

Occupational Cancers

Research Project Grants (R01)

(01331) A Carcinogen/Mutagen Detector for HPLC 55
(02027) Production and Fate of Hydroxamic Acids in Hepatocytes 57
(02067) Occupational Cancer Surveillance: New Approaches 59
(02104) A Petroleum Solvent Mortality Study of Dry Cleaners 61
(02122) Urinary Excretion of Modified Nucleosides 63
(02284) Feasibility Study for an Occupational Cancer Data Base 64
(02351) Factors Modifying Diesel Particulate Composition 65
(02413) Detection of Adriamycin on Surfaces and Skin 67

Small Grants (R03)

(02238) Development of a Human DNA Adduct Monitoring Method 68
(02243) Cytogenetic Assessment of Occupational Exposure to Antineoplastic Agents 69

Traumatic Injuries

Research Project Grants (R01)

(01122) Explosion Hazards Related to Grain and Feed Dusts 70
(02241) Work-Related Burn Injury Incidence in New England 72
(02254) An Epidemiologic Study of Injuries in Firefighters 73

Disorders of Reproduction

Research Project Grants (R01)

(00835) Occupational Risks of Pesticide Exposure for Females 74
(01520) Reproductive Hazards of Dinitrotoluene and Toluenediamine 76
(01910) Reproductive Effects of Lead or Solvent Exposure at Work 78
(02148) Effects of 27 MHz Radiation on Somatic and Germ Cells 80
(02191) Environmental/Industrial Toxicants and Testicular Injury 83

Career Development Grants (K01)

(00035) Genotoxic Exposure Assessment by Simplified DNA Analysis 85
(00059) Role of Gap Junctions in Adverse Reproductive Outcome 87

Small Grants (R03)

(01827) Occupational Factors Associated with Biliary Atresia 88
(02258) Role/Epididymal Glutathione in Chemical-Induced Germ Cell Mutation 90
(02345) Male Reproductive Effects of Acrylamide 91
(02376) Prenatal Lead Exposure and Skeletal Growth 93
(02380) Disorders of Reproduction Among Female Veterinarians 95
(02383) Menstrual Function In Nurses Exposed to Cancer Drugs 96
(02548) Adverse Pregnancy Outcomes Among Cosmetologists 97

Neurotoxic Disorders

Research Project Grants (R01)

(00823) Occupational Neuropathies Due to Industrial Chemicals 98
(00952) Chronobiology and Occupational Health Hazards 101
(01972) Molecular Mechanisms of Diketone Neurotoxicity 104
(02003) Mechanisms of Occupational Neuropathies 106
(02020) Acrylamide Neurotoxicity: Role of Oxidative Metabolism 108
(02065) Mechanisms of Occupational Distal Axonopathy 110
(02384) Validation of a Neurobehavioral Test Battery 112

Career Development Grants (K01)

(00064) Peripheral Nervous Effects of Workplace Neurotoxins 113

Small Grants (R03)

(02095) Test for Neurotoxins Using Caenorhabditis Elegans 115

Noise-Induced Hearing Loss

Research Project Grants (R01)

(01152) The Effects of Impulse Noise on the Auditory System 116
(02128) Functional Correlates of Cochlear Injury 118
(02317) Hearing Hazard Associated with Industrial Noise Exposure 120

Career Development Grants (K01)

(00042) Noise-Induced Hearing Loss and High Blood Pressure 123

Small Grants (R03)

(02141) Auditory Temporal Acuity Following Noise Exposure 125

Small Business Grants (R43, R44)

(02313) Audio Mechanical Transducer Communication System 126

Dermatologic Conditions

Research Project Grants (R01)

(02021) A Personal Dosimeter for Exposure to Ultraviolet Light 127
(02091) Immunotoxicology of Phenols on Epidermal Immune Cells 128

Career Development Grants (K01)

(00017) Cutaneous Exposure: Predictive Pathways 130

Small Grants (R03)

(01990) Occupation Health Hazards in Skin Injury by Mitomycin C 133
(02433) Mechanisms of Cytotoxicity by Chlorodinitrobenzene, A Potent Sensitizer 135

Psychological Disorders

Research Project Grants (R01)

(01968) Occupational Stress and Health of Women LPN's and LSW's 136
(02024) Impact of Automation on Industrial Inspector Stress 138
(02162) Job and Family Stress and Women's Work Performance 140

Career Development Grants (K01)

(00028) Neuropsychological Effects of Chronic Solvent Exposure 141

Engineering Control Systems

Research Project Grants (R01)

(01932) A Model for Predicting Glove Polymer Permeation 143
(02132) Fundamental Investigation of Exhaust Hoods 145
(02329) Ventilation for Work in Confined Space 147
(02392) The Impact of Separation on Exposure and Hood Capture 149

Small Grants (R03)

(02101) Capture Efficiency of Local Exhaust Ventilation Systems 150
(02236) Tractor Stability Information Processing System 151
(02422) Reducing Solvent Exposure of Auto Body Workers 152

Small Business Grants (R43, R44)

(01951) Noise Control for Honing and Other Machines/Phase II 153

Respirator Research

Research Project Grants (R01)

(01301) New Methods for Quantitative Respirator Fit Testing 154
(01485) A Fundamental Study of Respirator Air Filtration 155
(01595) Respirator Performance Model for Particulates 157
(01603) In-Mask Monitors and Respirator Breakthrough 159
(01632) Measure of Work Performance Decrement Due to Respirators 160
(01644) Optimal Design of Respirator Cartridges 161
(01646) Model and Tests for Prediction of Respirator Service Life 163
(02005) Respirator Tolerance 165
(02154) Asbestos Fiber Collection by NIOSH-Approved Respirators 166

Career Development Grants (K01)
(00068) Quantitative Respirator Fit Test by Negative Pressure 167
Small Business Grants (R43, R44)
(02312) An Advanced Respiratory Protective Device 168

Other Occupational Concerns
Research Project Grants (R01)
(00978) Human Metabolism of Halothane-Mechanisms of Toxicity 170
(01630) Chromium Distribution and Toxicity in Mammalian Cells 172
(01981) Poison Centers: A Resource for Occupational Health Services 175
(02066) Improved Personal Protection at Hazardous Waste Sites 177
(02108) Polyimide Sorbents for Airborne Toxic and Carcinogens 179
(02149) Plasma Proteins: Markers of Chemical Exposure 181
(02214) Environmental Toxicity of Isocyanates 183
(02221) Dose/Response for Occupational Styrene Exposures 185
(02288) Artificial Intelligence Occupational History 187
(02303) Fast-GC for Industrial Hygiene Monitoring/Analysis 188
(02404) Self-Training, Self-Optimizing Infrared Expert System 189
(02417) Halogenated Hydrocarbon Toxicity in Proximal Tubules 190
(02586) Effects of Job Hazards, Health, Incentives on Absenteeism 191
Career Development Grants (K01)
(00054) Peripheral Markers of Muscarinic Receptors 192
(00055) Highway Maintenance Cohort Mortality Study 194
(00063) Minimizing Dermal Exposure to Pesticides in Greenhouses 196
(00071) Hepatic Steatosis and Solvents: A Case-Control Study 197
Small Grants (R03)
(01825) Anemometry Related to Workplace Contaminant Distribution 198
(02044) Size Distribution of Airborne Lead Aerosol in Industry 200
(02179) SCBA Stressors: Effects of Carbon Dioxide Breathing 201
(02180) Measurement of Worker's Exposure to Styrene 203
(02321) Mechanisms of Cytoskeletal Injury by Heavy Metals 205
(02375) Development of Methods to Estimate Berryllium Exposure 207
(02377) Formaldehyde Molecular States in the Gas Phase 208
(02553) Quantifying Professional Judgment in Industrial Hygiene 210

GRANTS ACTIVE DURING FY87 211

FY87 GRANT AWARDS BY PROGRAM AREA 212

FY87 GRANT AWARDS BY REGION AND STATE 213

GRANT NUMBER INDEX BY GRANT ACTIVITY 214

PRINCIPAL INVESTIGATOR INDEX BY GRANT ACTIVITY 217

GRANTEE INSTITUTION INDEX BY GRANT ACTIVITY 220

INTRODUCTION

The organization of this annual report on the NIOSH research grants program is designed to facilitate the reader's understanding of the types of extramural research projects supported under the primary areas of NIOSH's interest with respect to the leading work-related diseases and injuries.

- Summaries of the supported projects are grouped according to these major areas of interest, as indicated in the *Table of Contents*
- Within each program area, projects are grouped by type of grant (e.g., research project grant, career development grant, and small grant).

> **Note:** *See the program announcement beginning on page 2 for descriptions of these grant types and other types that NIOSH awards.*

Each grant summary contains administrative information about the grant, followed by a synopsis of the project and any publications that have resulted to date.

- There are two possible formats for a synopsis. Principal investigators are given the choice of which format to use, and they prepare the summaries for inclusion in this report.
  - One format is simply the abstract from the grant application.
  - The other format is a more complete explanation of the nature of the project and a discussion of results, with sections on *Objectives, Methodology, Progress and Accomplishments, and Significance.*
- Publications are listed so that the reader may gain more information about the projects than is given in the brief summaries. Although some citations are not yet published or may not be retrieved easily, they have been included for the sake of providing maximum information.

> **Note:** *Should there be an interest in more information, principal investigators should be contacted directly.*

Statistics on the number and amount of funds awarded by grant type, program area, and region/state are given in tabular form at the end of the report. Also included are indices for ease in locating particular grants if the reader knows the grant number, the principal investigator, or the grantee institution.

> **Note:** *See glossary on page 9 for an explanation of the components of a grant number.*

Suggestions on content or format of this report to make it more useful to the reader would be welcomed. The process of assembling the report begins in the fall of each year, so comments should be received at least by the end of September.

- Inquiries or ideas should be addressed to:

NIOSH Grants Office
1600 Clifton Road
Building 1, Room 3053
Atlanta, Georgia 30333
404/639-3343

ANNOUNCEMENT

RESEARCH AND DEMONSTRATIONS RELATING TO OCCUPATIONAL SAFETY AND HEALTH

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

Application Receipt Dates: New applications (February 1, June 1, October 1)
Exceptions: Career Development, Small Grants, and Competing renewal applications (March 1, July 1, November 1).

The Centers for Disease Control (CDC), National Institute for Occupational Safety and Health (NIOSH) announces that competitive grant applications are being accepted for research and demonstrations relating to occupational safety and health. These include innovative methods, techniques, and approaches for dealing with occupational safety and health problems in the general industry and in the mining industry.

Support in the form of individual (research, demonstration, and program) project grants will be awarded for annual budget periods, within a project period not to exceed five years.

I. AUTHORITY

These grants will be awarded and administered by NIOSH under the research and demonstration grant authority of Section 20(a)(1) of the Occupational Safety and Health Act of 1970 (29 U.S.C. 669(a)(1) and Section 501(c) of the Federal Mine Safety and Health Amendments Act of 1977 (30 U.S.C. 951). Program regulations applicable to these grants are contained in Part 87 of Title 42, Code of Federal Regulations, "National Institute for Occupational Safety and Health Research and Demonstration Grants." Except as otherwise indicated, the basic grant administration policies of the Department of Health and Human Services and the Public Health Service are applicable to this program.

II. ELIGIBLE APPLICANTS

Eligible applicants include non-profit and for-profit organizations. Thus universities, colleges, research institutions and other public and private organizations including State and local governments and small, minority and/or woman-owned businesses are eligible for these research and demonstration grants. For-profit organizations will be required to submit a certification as to their status as part of their application.

III. PROGRAM REQUIREMENTS

A. Research Project Grants

A research project grant application should be intended and designed to establish, discover, develop, elucidate, or confirm information relating to occupational safety and health, including innovative methods, techniques, and approaches for dealing with occupational safety and health problems. These studies may generate information that is readily available to solve problems or contribute to a better understanding of underlying causes and mechanisms.

B. Demonstration Grants

A demonstration grant application should address, either on a pilot or full-scale basis, the technical or economic feasibility or application of: (1) a new or improved occupational safety or health procedure, method, technique, or system, or (2) an innovative method, technique, or approach for preventing occupational safety or health problems.

C. Special Emphasis Research Career Award (SERCA) Grants

The SERCA is designed to enhance the research capability of individuals in the formative stages of their careers who have demonstrated outstanding potential for contributing as independent investigators to health-related research. Candidates must have had two or more years of relevant postdoctoral experience prior to the submission date. The application must document accomplishments in this period that demonstrate research potential; it must also present a plan for additional experience in a productive scientific environment at domestic institutions that will foster development of a career of independent research in the area of occupational safety and health. The SERCA is not intended for untried investigators, or for productive, independent investigators with significant numbers of publications of high quality, or for persons of senior academic rank (above associate professor or tenured). Moreover, the award is not intended to substitute one source of salary support for another for an individual who is already conducting full-time research, nor is it intended to be a mechanism for providing institutional support. The application must demonstrate that the award will make a difference in and enhance the candidate's development as an independent investigator.

Candidates must indicate a commitment of at least 60 percent time (not necessarily 60% salary) devoted to research under the SERCA grant, although full-time is desirable. Other work in the area of occupational safety and health will enhance the candidate's qualifications but is not a substitute for this requirement. While working closely with one or more advisers, the awardee is expected to develop capabilities in fundamental, applied, and/or clinical research in one of the areas in section IV. At the end of the award period, evidence of independent investigative capability should be present such that the individual is better able to compete in traditional NIOSH research grant activities.

The total grant award may comprise direct costs of up to $30,000 per year and up to eight percent additional indirect costs. Direct costs may include salary plus fringe benefits, technical assistance, equipment, supplies, consultant costs, domestic travel, publication, and other costs. If the awardee already holds a small grant on the same research topic, the amount of the SERCA may be reduced up to the amount of the small grant. Awards may be up to three years and will not be renewable.

D. Small Grants

A small grant application is intended to provide financial support to carry out exploratory or pilot studies, to develop or test new techniques or methods, or to analyze data previously collected. This small grant program is intended for predoctoral graduate students, post-doctoral researchers (within three years following completion of doctoral degree or completion of residency or public health training) and junior faculty members (no higher than assistant professor). If university policy requires that a more senior person be listed as principal investigator, the application should specify that the funds are for the use of a particular student or junior-level person and should include appropriate justification for this arrangement. Though biographical sketches are required only for the person actually doing the work, the application should indicate who would be supervising the research. Small grant applications should be identified as such on the application form.

The total small grant award may comprise direct costs of up to $15,000 per year and additional indirect costs, as appropriate. The grants may be awarded for a project period of up to two years and are thereafter continuable by competitive renewal as a regular research grant. Salary of the principal investigator as well as that of the junior investigator, if university policy requires a senior person to be listed as the principal investigator, will not be allowed on a small grant, though salaries can be requested for necessary support staff such as laboratory technicians, interviewers, etc.

E. Program Project Grants

NIOSH will also accept applications for program project grants, but only after discussion with the individuals listed in this announcement. A program project grant is intended to support a broadly-based, multidisciplinary, often long-term research program which has a specific major objective or a basic theme. It should be directed toward a range of problems having a central research focus in contrast to the usually narrower thrust of the traditional research project. This type of grant generally involves the organized efforts of a group of established investigators, each of whom is conducting research projects designed to elucidate the various aspects or components of the overall objective.

IV. PROGRAMMATIC INTEREST

NIOSH program priorities, listed below, are applicable to all of the above types of grants. The conditions or examples listed under each category are selected examples, not comprehensive definitions of the category. Investigators may also apply in other areas related to occupational safety and health. Applications responding to this announcement will be reviewed by staff for their responsiveness and relevance to occupational safety and health. Assignment to NIOSH for funding consideration will be according to established referral guidelines. Potential applicants with questions concerning the acceptability of their proposed work should contact the individuals listed in this announcement under **FOR FURTHER INFORMATION CONTACT**.

1. Occupational lung diseases: asbestosis, byssinosis, silicosis, coal workers' pneumoconiosis, lung cancer, occupational asthma

2. Musculoskeletal injuries: disorders of the back, trunk, upper extremity, neck, lower extremity; traumatically induced Raynaud's phenomenon

3. Occupational cancers (other than lung): leukemia; mesothelioma; cancers of the bladder, nose and liver

4. Severe occupational traumatic injuries: amputations, fractures, eye loss, and lacerations

5. Cardiovascular diseases: hypertension, coronary artery disease, acute myocardial infarction

6. Disorders of reproduction: infertility, spontaneous abortion, teratogenesis

7. Neurotoxic disorders: peripheral neuropathy, toxic encephalitis, psychoses, extreme personality changes (exposure-related)

8. Noise-induced hearing loss

9. Dermatologic conditions: dermatoses, burns (scalding), chemical burns, contusions (abrasions)

10. Psychological disorders: neuroses, personality disorders, alcoholism, drug dependency

11. Engineering control systems: new technology performance evaluation, preconstruction review, equipment redesign, containment of hazards at the source, fundamental dust generation mechanisms, machine guarding/avoidance methods, explosion control, removal of emissions after generation, dispersion models, monitoring and warning techniques, technology transfer

12. Respirator research: new and innovative respiratory protective devices; techniques to predict performance; effectiveness of respirator programs; physiologic and ergonomic factors; medical surveillance strategies; psychological and motivational aspects; Effectiveness of sorbents and filters, including chemical and physical properties

V. CRITERIA FOR REVIEW

Applications will be evaluated by a dual review process. The primary (peer) review is based on scientific merit and significance of the project, competence of the proposed staff in relation to the type of research involved, feasibility of the project, likelihood of its producing meaningful results, appropriateness of the proposed project period, adequacy of the applicant's resources available for the project, and appropriateness of the budget request. A program project application will also be evaluated for adequacy of methods for coordinating activities toward the central focus.

Demonstration grant applications will be reviewed additionally on the basis of the following criteria:

- Degree to which project objectives are clearly established, obtainable, and for which progress toward attainment can and will be measured.

- Availability, adequacy, and competence of personnel, facilities, and other resources needed to carry out the project.

- Degree to which the project can be expected to yield or demonstrate results that will be useful and desirable on a national or regional basis.

- Extent of cooperation expected from industry, unions, or other participants in the project, where applicable.

SERCA grant applications will be reviewed additionally on the basis of the following criteria:

- The review process will consider the applicant's scientific achievements, evidence of demonstrated commitment to a research career in occupational safety and health, and supportive nature of the research environment (including letter(s) of reference from advisor(s) which should accompany the application).

Small grant applications will be reviewed additionally on the basis of the following criteria:

- The review process will take into consideration the fact that the applicants do not have extensive experience with the grant process.

A secondary review will also be conducted. Factors considered in the secondary review will include:

- The results of the initial review.
- The significance of the proposed study to the research programs of NIOSH.
- National needs and program balance.
- Policy and budgetary considerations.

VI. APPLICATION AND AWARD

Applications should be submitted on Form PHS-398 (revised September 1986) or PHS-5161-1 for State and local government applications. Forms should be available from the institutional business offices or from:

Office of Grants Inquiries
Division of Research Grants
National Institutes of Health
Westwood Building - Room 449
5333 Westbard Avenue
Bethesda, Maryland 20205

The original and six copies of the application must be submitted to the address below on or before the specified receipt dates in accordance with the instructions in the PHS-398 packet:

Division of Research Grants
National Institutes of Health
Westwood Building - Room 240
Bethesda, Maryland 20205

In developing the application please note that the conventional presentation for grant applications should be used and the points identified under **CRITERIA FOR REVIEW** must be fulfilled.

An applicant organization has the option of having specific salary and fringe benefit amounts for individuals omitted from the copies of the application that are made available to outside reviewing groups. If the applicants organization elects to exercise this option, use asterisks on the original and six copies of the application to indicate those individuals for whom salaries and fringe benefits are being requested; the subtotals must still be shown. In addition, submit an additional copy of page four of Form PHS-398, completed in full with the asterisks replaced by the amount of the salary and fringe benefits requested for each individual listed. This budget page will be reserved for internal PHS staff use only.

The instructions in the Form PHS-398 packet should be followed concerning deadlines for either delivering or mailing the applications. The application should be sent or delivered using the mailing label in the Form PHS-398 packet.

The proposed timetable for receiving applications and awarding grants is as follows except for the SBIR Program, which has a separate Public Health Service announcement and a separate receipt date. Applications for the SBIR Program have a receipt date of December 15.

New and competing renewal applications:

Application Deadline*	Primary Review Group Meeting	Secondary Review Meeting	Expected Start Date
February 1	June	September	December 1
June 1	Oct./Nov.	January	April 1
October 1	Feb./Mar.	May	July 1

* Competing renewal deadlines are one month later.

Exceptions: Career Development and Small Grants

Application Deadline	Primary Review Group Meeting	Secondary Review Meeting	Expected Start Date
March 1	June	September	December 1
July 1	Oct./Nov.	January	April 1
November 1	Feb./Mar.	May	July 1

Awards will be made based on priority score ranking and emphasis area, as well as availability of funds.

VII. REPORTING REQUIREMENTS

Performance reports on awarded grant projects are required annually as a part of the continuation application and a final report is due within ninety days of the end of the project period. The final performance report should include, at a minimum, a statement of original objectives, a summary of research methodology, a summary of positive and negative findings, and a list of publications resulting from the grant. Research papers, project reports, or theses are acceptable items in the final report. The report should stand alone rather than citing the original application. Three copies of reprints of publications prepared under the grant should accompany the report.

VIII. FOR FURTHER INFORMATION CONTACT:

For Technical Information Contact:

Roy M. Fleming, Sc.D.
Associate Director for Grants
National Institute for Occupational Safety and Health
Centers for Disease Control
1600 Clifton Road, N.E., Bldg. 1, Room 3053
Atlanta, Georgia 30333
Telephone: (404) 639-3343

For Business Information Contact:

Henry S. Cassell
Grants Management Officer
Centers for Disease Control
255 E. Paces Ferry Rd., NE, Room 321
Atlanta, Georgia 30305
Telephone: (404) 842-6575

(This program is described in the Catalog of Federal Domestic Assistance Program No. 13.262, Occupational Safety and Health Research Grants. It is not subject to Health Systems Agency nor E.O. 12372 review.)

HHS,PHS,CDC - 5/88

GLOSSARY

Grant Number - The identification number assigned to a grant application

EXAMPLE: 1 R01 OH02183-01A1

```
1  R01  OH  02183  -01  A1
|   |    |    |     |    |
|   |    |    |     |    |_ Signifies a first resubmission with modifications
|   |    |    |     |
|   |    |    |     |_ Year of funding
|   |    |    |
|   |    |    |_ Sequence number for NIOSH grants
|   |    |
|   |    |_ Institute with primary assignment
|   |
|   |_ Grant Activity
|
|_ Grant Type
```

Typical Codes:

Grant Type

1 - New Competing application
2 - Competing renewal application
5 - Noncompeting continuation application

Grant Activity

R01 - Research Project Grant
K01 - Special Emphasis Research Career Award (SERCA) Grant
R03 - Small Grant
R13 - Conference Grant
R18 - Demonstration Grant
R43 - Small Business Innovation Research (SBIR) Grant

Institute

OH - NIOSH

Program Areas - Research categories of particular interest to NIOSH

Occupational Lung Diseases	(Lung)
Musculoskeletal Injuries	(Muscu)
Occupational Cancers	(Cancer)
Traumatic Injuries	(Trauma)
Cardiovascular Diseases	(Cardio)
Disorders of Reproduction	(Repro)
Neurotoxic Disorders	(Neuro)
Noise-Induced Hearing Loss	(Noise)
Dermatologic Conditions	(Derma)
Psychological Disorders	(Psycho)
Engineering Control Systems	(Contr)
Respirator Research	(Respir)
Other Occupational Concerns	(Other)

Aerosol Deposition in the Human Respiratory System

C. P. Yu, Ph.D.
State University of New York at Buffalo
Department of Mechanical and Aerospace Engineering
Clifford C. Furnas Hall
Amherst, New York 14260

Occupational Lung Diseases
5 R01 OH00923-05
09/30/83 - 11/30/86
$58,351 ($215,528 Cum)

Objectives

This research is directed toward determining the amount and site(s) of deposition of inhaled particles and their subsequent fast clearance in the human respiratory system with the use of theoretical and computational models. Special efforts will be made to examine (i) the effects of various particle factors such as size, shape, charge, mass density and hygroscopicity on deposition and (ii) the extent and causes of intersubject variabilities.

Methodology

Analytical and statistical methods will be employed to achieve the goals with extensive reference to available experimental data. Computer programs are to be developed for predicting deposition under various exposure conditions.

Progress and Accomplishments

At this time, we have completed the following major studies:

1. Modeling of head deposition for spherical and fibrous particles.
2. Development of lung deposition models involving:
    a. derivation of deposition efficiency formulas in an airway due to various mechanisms;
    b. Study of particle charge effects;
    c. deposition of ultrafine particles;
    d. deposition of fibrous particles;
    e. deposition of hygroscopic particles;
    f. deposition in young age groups.
3. Development of probabilistic deposition models for:
    a. deposition of polydisperse aerosols;
    b. lung deposition in different anatomical models;
    c. statistical deposition models with random airway structures.
4. Determination of mucociliary transport rates in bronchial airways and the effect of sulfuric acid on such rates.

Significance

Airborne toxic particulates, inhaled into the respiratory system, will deposit there and produce detrimental biological effects. Since the complete removal of these particulates from the industrial environment is practically impossible and economically unfeasible, it is necessary to establish certain exposure standards for occupational protection. It is hoped that within these standards, any biological damage would be unlikely even after long periods of exposure. The results of this research will lead to a better fundamental understanding of the dose-response relationship for occupational-induced lung diseases and thus may serve as a basis for developing an effective prevention strategy.

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Health Risks in the Vermont Granite Industry

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Occupational Lung Diseases
5 R01 OH01035-04
04/01/83 - 03/31/87
$287,892 ($1,063,769 Cum)

Objectives

The overall aim of this study is to carry out a comprehensive assessment of health risks in the Vermont granite industry, which involves exposure to low levels of quartz. The major specific objectives are three:

1. To perform a longitudinal study of pulmonary function losses in the current work force. Previous estimates of losses suggested that the loss of forced vital capacity and FEV1 in this group of workers was excessive and was related to occupational dust exposure. Certain doubts were raised about these previous studies and the current effort is an attempt to confirm, deny, or qualify the suggestion that excessive loss occurs independent of smoking or aging.
2. The second objective is to carry out an industry wide radiographic survey to ascertain the prevalence of abnormalities consistent with pneumoconiosis in workers who have been exposed to low levels of granite dust since 1940. Radiographic surveys of the industry have been done by the Vermont Industrial Hygiene Division since 1940, suggesting as of 1965 that silicosis was being controlled, but studies published in the 1970's stated that a 30% prevalence of abnormalities existed. The current effort is to assess whether radiographic silicosis or silicotuberculosis exist and to what degree. Many of the workers studied will have a prolonged exposure to granite dust (more than 30 years). Those workers showing abnormalities will be compared with their normal colleagues in terms of smoking history and dust exposure to ascertain the significance of the radiographic changes.
3. The third aim is to make systemic measurements of dust levels in different size stone sheds and in different occupational categories within the industry.

A subsidiary objective includes a study of retired granite workers both with and without radiographic silicosis who have been followed longitudinally both by x-ray and pulmonary function testing. The purpose of this aspect of the project is to ascertain the rate of which pulmonary function changes occur in men who are no longer occupationally exposed to dust and to compare rates of pulmonary function decrement in those with and without silicosis.

Methodology

For spirometric testing, we have used a Collins 13 liter water-sealed Stead-Wells type apparatus since 1979. Testing is carried out at the workplace. Guidelines set down by the American Thoracic Society are being followed in terms of evaluating the results and the acceptability of tracings.

Collection of 8-hour dust samples has utilized personal size selective samplers running at 2 liters per minute for gravimetric estimation. The samples are being analyzed for percentage quartz using x-ray diffraction techniques.

Chest x-rays are being read by three certified B readers using the ILO/UC classification system. The definition of abnormality we propose to use is based on a profusion reading of 1/0 or greater of densities considered to be consistent with pneumoconiosis by either two or three of the readers. These are of course analyzed as to type of abnormality and location as prescribed by the format.

Progress and Accomplishments

Spirometric surveys have now been carried out four times, including two industry wide surveys which antedated the beginning of the project, which were done in 1979 and 1981. Subsequent surveys have been done in the Spring of 1983, 1985 and 1987. Participation of the work force appears to be high. These results have not been analyzed as yet longitudinally, though the data has been entered in the computers and is nearly ready for analysis. Approximately 450 dust samples have been collected in various sheds and assorted occupations. The average dust level is 581 $\mu g/m^3$; the range of dust exposure varies from 10 to over 1000 μg/m3. These values are comparable to those observed by previous investigators and by the Vermont Occupational Safety and Health Administration. Depending on occupational category, the average values for total dust varied from a low of 389 $\mu g/m^3$ for boxers to a high of 912 $\mu g/m^3$ for maintenance workers (N-6). X-ray diffraction results suggest that the quartz content of the dust is 22%, rather than the 9-11% measured by previous workers using the IR technique. This means that a majority of the work force is being exposed to levels higher than the current OSHA limits.

The roentgenographic survey carried out in 1983 numbered approximately 970 workers, and this material has been interpreted and analyzed. Only 28 films were considered to be abnormal as defined by 2 or 3 readers seeing opacities consistent with pneumoconiosis in a profusion of 1/0 or greater. However, 22 of these were abnormalities of the irregular or reticular type and appeared to be predominantly lower lobe. This low prevalence occurred in the face of high dust exposures.

In addition, we have either x-rayed recently or found films on an additional 1000-2000 former or retired workers who were no longer employed during the survey of 1983. These films are being interpreted and coded. The total x-ray analysis should include close to 90% of all Vermont stone-shed workers employed since 1940 and exposed to low dust levels.

Standardized symptom questionnaires have been administered to granite workers and two comparison groups. The granite workers appear to have more phlegm and cough than the highway workers, but less chest illness and shortness of breath. There are a number of confounding variables, such as smoking, education and age, making the analysis complex.

Significance

The usefulness of this information pertains mainly to the issue of a safe occupational level of quartz exposure. Currently, the Department of Labor accepts a level of 100 $\mu g/m^3$ of quartz, whereas NIOSH has recommended a level of 50 $\mu g/m^3$. The levels of dust that we have measured are certainly above the NIOSH recommended level and may be considerably higher than the current levels, based on our estimate of 22% quartz using x-ray diffraction classification. It appears that the level of radiographic silicosis is extremely low, and there appeared to be no large opacities suggesting complicated disease nor any tuberculosis. The issue whether contemporary dust levels are leading to an excessive loss of pulmonary function should be possible to extract from the data since we will have internal controls. Thus, we will be able to compare granite workers exposed to a given level of dust and of comparable lengths of exposures among smokers and non-smokers.

Experimental Pneumoconiosis of Sandblasting Substitutes

Occupational Lung Diseases
5 R01 OH01970-03
04/01/84 - 03/31/87
$41,644 ($214,128 Cum)

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Objectives

Sandblasting is associated with severe and rapidly progressive silicosis. Recognition of these hazards led to its prohibition in Australia and Europe; in the U.S., sand substitutes are now increasingly being used. They are derived from coal ash residues and slag from smelting furnaces and are comprised of amorphous silicates of Fe, Al, Ca, Mg, and Ti. The free silica content is only 0.03%. The objective of this project is to develop a rat model to assess the pulmonary fibrogenic potential of sand substitutes. Both normal and silicotic rats are investigated after exposure to the substitutes. After exposure, both groups are followed for an additional 12 months. Control and exposed rats are sequentially sacrificed for histologic and tissue mineral content evaluations as well as biochemical assessment of hydroxyproline (HP) lung content.

Methodology

The first group of rats is exposed by inhalation to a ball-milled dust of the substitute at a concentration of 10 mg/m^3 for 6 hrs/day, 5 days/week for 12 months. Silicosis is induced in a second group by 3 intratracheal instillations, each consisting of 0.833 mg silica dust suspended in 0.3 ml saline (total dose of 2.5 mg). Nine months after instillation, these animals are also exposed by inhalation to the substitute dust (same dust concentration as the first group) for 6 months. Rats are evaluated for: (1) lung mineral burden; substitute dust determined by ICP and silica dust by X-ray diffraction, (2) HP content of lung tissue; and (3) histology; evaluation by light microscopy of sagital sections of right and left lung lobes using hematoxylin and eosin, Gomoris' silver impregnation, Masson's trichrome and Harrow Viche stains.

Progress and Accomplishments

Exposure was conducted in a whole body inhalation exposure system (64 rats). The coefficient of variation (CV) of dust retention in the lungs was about 18% (10 rats). A new, simple and consistent method for intratracheal instillation of silica dust was developed and evaluated. The amount of silica dust retained in the lungs was 80% of the injected amount with a CV of 13.5% (6 rats). Analysis of original substitute and ball-milled dust by the ICP method showed that ball-milling did not change the chemical composition of the starting material. Histologic examination of rat lungs exposed to Stanblast dust for 6 and 12 months displayed small, discrete, cellular accumulations profusely scattered throughout the parenchymal field. They were most often noted in peribronchiolar, perivascular and subpleural location. The majority of these cellular collections were composed of large alveolar macrophages and, less frequently, lymphocytes. Polymorphonuclear leukocytes were rarely seen. Stanblast particles were readily apparent in these locations both intra-and extracellularly. The particulates were numerous and appeared to completely obliterate the cellular components within the foci. Examination of the alveolar walls surrounding these nodules revealed individual interstitial macrophages containing granules of Stanblast material. Stanblast burdened cells were also frequently seen within the peribronchiolar lymphoid tissues. In addition to these benign appearing lesions, a very small proportion of the foci, observed in the 12-month exposed rats, were dominated by a lymphocytic infiltrate (rather than alveolar macrophages), and displayed an apparent increase in eosinophilic ground substance, suggestive of early granulomatous formation. Assessment of collagen deposition within these foci utilizing the Harrow Viche stain did not reveal a significant increase in interstitial collagen. These variants of Stanblast lymphocyte collections were noted in 4 of the 10 animals exposed for 12 months. Histologic features observed in animals sacrificed 6 and 12 months after one year of exposure were similar. Biochemical evaluations corroborated the histologic findings. HP contents of the exposed rat lungs were not significantly different from the controls at 6, 12, 18 and 24 months from onset of exposure. Evaluation of silicotic rat lungs' reaction to the substitutes is currently in progress.

Significance

This study will assess for the first time the safety of these substitutes. It is believed that this rat model will have application in delineating and predicting the potential occupational hazard for abrasive blasters with and without pre-existing silicosis. Occupational exposure standards are based on the assumption that the risk of any particular agent is not influenced by exposure to other environmental factors. This study will provide important information on the risk of lung fibrosis from biologic interactions between different inhaled agents, which could affect the future development of occupational standards. More specifically, this project may demonstrate increased risk of progression of silicosis if silicotic sandblasters use the substitutes.

Respiratory Hazards of Poultry Workers

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Occupational Lung Diseases
5 R01 OH02029-04
09/01/84 - 08/31/88
$ 70,038 ($601,726 Cum)

Objectives

We propose the first indepth medical and environmental study to investigate possible occupational hazards of poultry workers. Our research has four major aims:

1. To characterize the operations within the various components of the poultry industry on a national basis, relative to occupational health risks.
2. To identify the significant subgroups of poultry workers at potential risk.
3. To evaluate the air in the work environment of selected establishments for potentially harmful vapors and dusts.
4. To make any indicated recommendations to the poultry industry for improvement of the workplace and worker protection.

Methodology

The proposed investigation is a three-phase four-year study. Phase I is a 12-month effort to develop an industry-wide worker exposure profile by performing a series of walkthrough evaluations of typical turkey-growing, chicken-growing, hatcher, egg production, and poultry processing operations. We intend to identify and quantify the subgroups of poultry workers with the greatest potential for occupational health risks for comprehensive medical and environmental studies in phase II.

In phase II (years 2 and 3) we will recruit 400 workers for medical studies, (100 workers from each of the 4 subgroups of poultry workers at greatest risk). Medical assessment of these subjects will include ATS questionnaire (modified for occupational history), spirometry before and after 2 hours of work, serum precipitins, and allergy skin testing. These data will be compared to two reference populations: 1) regionally proportioned unexposed blue collar workers, and 2) a blue collar reference population previously studied by NIOSH. Environmental assessment of the estimated 116 work-sites and 20 control work-sites will include measurements for ammonia, carbon dioxide, carbon monoxide, total and respirable dust, and endotoxin.

Phase III, data analysis, will occur during year 04 of the study. We will examine the relationships of environmental exposures to respiratory symptoms and pulmonary function. We will also examine relationships of environmental control systems and levels of environmental contaminants. We will determine what recommendations are needed if any, to improve the work environment, to protect workers from potential health hazards, and for possible future research.

Progress and Accomplishments

Phase I of the project includes both data collection and data generation activities. A comprehensive literature review has been underway and is continuing. This review involves production figures, processes, structures, work practices, a general assessment of potential occupational exposures, and geographical influences on these characteristics. The goal of this phase is to determine the size of the population at risk and to get an idea of air contaminants that may be encountered in phase II.

Walkthrough assessments of poultry operations in Iowa, Minnesota, Arkansas, and California have been completed. Data has been collected concerning concentration of airborne dust and ammonia. Types of building design with the inherent variables of ventilation and bird handling have been ascertained. With these data in hand a risk index is being developed to qualify those groups with greatest exposure and potential occupational hazards.

The second phase of this study involved collection of data from subjects in the following groups: 90 subjects at 57 laying operations, 97 subjects at 41 turkey growing operations, 28 poultry loaders at 7 sites, and 32 shacklers at 3 processing sites. In addition to studying these 247 exposed workers, we performed similar tests on 144 non-exposed blue collar workers at 3 sites. Their data - both medical and environmental assessments - will be compared to that of exposed workers. Plans have been made to study 50-100 broiler growers in Oklahoma at 17 sites in January 1988, and will conclude data collection on the project. The remainder of year 4 will be spent analyzing medical and environmental data gathered in years 2, 3 and 4.

Significance

Agricultural workers are a large and understudied population. Over the past few years we have recognized an emerging occupational respiratory disease hazard to farmers and farm workers within swine confinement buildings. Since most of the poultry in the U.S. are now raised in confinement buildings, we anticipate similar occupational problems may be present in poultry workers. Our pilot studies suggested they experience exposures and respiratory symptoms similar to swine confinement workers.

Publications

Leistikow B, Pettit W, Donham KJ, et al: Respiratory Risks from Poultry Farming. Health and Safety In Agriculture. Proc Intern'l Sym on Health and Safety in Agriculture, Saskatoon, Saskatchewan. October 8-11, CRC Press, 1987

Pulmonary Toxicity of the Semiconductor, Gallium Arsenide

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Occupational Lung Diseases
5 R01 OH 02076-03
11/01/84 - 10/31/88
$131,079 ($372,512 Cum)

Objectives

The objective is to investigate the lung toxicity and potential systemic toxicity of particulate gallium arsenide (GaAs), a semiconductor in industrial use. Previous work has shown a dose-dependent increase in rat lung weight after intratracheal GaAs that was not caused by aqueous edema.

The aims of this study are to define the lung changes induced by GaAs and to determine if there are lung indicators of toxicity which would be useful for Group III-Group V intermetallic semiconductor compounds. Specifically, the ability of GaAs to cause pulmonary fibrosis after a single intratracheal dose in an analogous manner to silica will be examined. This will be studied as a function of dose and will be compared with arsenic (III) oxide. Indicators of As(III) and As(V) systemic toxicity will be studied in four species to measure the species suitability for use in evaluating GaAs systemic toxicity.

Methodology

The response of the Spraque-Dawley rat to As(III) and As(V) will be compared to the B6C3F1 mouse, Golden Syrian hamster and the Hartley guinea pig. The doses will be 1.0 and 0.1 mg/kg administered by intraperitoneal injection and the parameters to be examined include: blood - hematocrit, total As, glucose, creatinine and urea nitrogen levels; kidney - pyruvate dehydrogenase activity and urine - total As, glucose, protein, creatinine, uroporphyrin and coproporphyrin.

The metabolism of gallium arsenide, sodium arsenite and sodium arsenate will be compared by quantifying the urinary metabolites after intratracheal administered of the three arsenic compounds to hamsters. The metabolites, As(III), As(V), mono methylarsonic acid and dimethylarsinic acid, can be separated by ion exchange chromatography and quantified by atomic absorption spectrophotometry.

The lung effects will be examined from the following parameters: wet weight, dry weight, total protein, total DNA, arsenic, 4-hydroxproline, phospholipids, cholesterol and lung histology The lung histology will be examined by light microscopy after H&E, reticulum and Masson's trichrome stains and by electron microscopy. Special immunofluorescence stains for fibronectin and collagen III will be examined.

Progress and Accomplishments

The species comparison experiment was completed. Large background levels of blood arsenic were found in rats. These results confirmed that As blood levels were unreliable as an indicator of exposure in rats and that rats should not be used for disposition studies. The hamster, mouse and guinea pig showed dose-dependent increases in As blood levels. Kidney pyruvate dehydrogenase (PHD) activity was about equally depressed for mouse, hamster and guinea pig after 1.0 mg/kg As as As (III) was administered intraperitoneally. The mouse also showed a decrease in PDH activity at 15 min for the 0.1 mg/kg dose. The rat showed a decrease in PDH at 8 and 24 hr after 1.0 mg/kg As(III), times much later than expected for PDH effects. When the same amount of As(V) was administered to rats and mice, the rat showed the lowest PDH activity at the 15 and 30 min time points and was still substantially inhibited at 1 hr. Only the mouse showed increased uroporphyrin and coproporphyrin levels for both As(III) and As(V) administration. No other changes were noted in any of the other parameters.

We conclude that the rat may be an appropriate model for systemic toxicity studies, particularly if As(V) exposure is suspected. Otherwise, the mouse is the most sensitive species of those tested.

The metabolites excreted in urine after GaAs intratracheal exposure were compared with As(III) and As(V). The same metabolites were found for all 3 arsenic species and the ratios of metabolites formed after GaAs were between those found for As(III) and As(V).

The lung toxicity was examined for dose levels of 2.5, 30 and 100 mg/kg GaAs, 1.7 and 17 mg/kg arsenic (III) oxide and 200 mg/kg DQ-12 silica at time points of 1, 2 and 4 weeks, 3 and 6 months after a single intratracheal dose. Early time points showed that 100 mg/kg GaAS showed similar lung inflammation and the effects were dose dependent for GaAs. The GaAs did not develop the nodular fibrosis like silica and most parameters returned to near normal. Microscopy indicated increased alveolar wall thickness and foamy macrophages at 6 months. The 2.5 mg/kg GaAs dose did not cause the lung changes.

Significance

These experiments have shown that GaAs does not have the same potential for occupational lung disease as silica after a single dose. There are lung changes and the potential for lung disease to GaAs results in arsenic being absorbed and metabolized like inorganic arsenic. Thus, there is the potential for systemic toxicity in addition to the lung changes.

Publications

Rosner MR, Carter DE: Metabolism and Excretion of Gallium Arsenide and Oxides by Hamsters Following Intratracheal Administration. Fundamental and Applied Toxicology 9: 730-737, 1987

Epithelial Surface Proteins: Markers of Cancer Risk

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Occupational Lung Diseases
5 R01 OH02114-02
01/01/86 - 12/31/89
$160,896 ($293,780 Cum)

Objectives

We planned to study alterations in the bronchial epithelium caused by exposure to cigarette smoke and asbestos. We have chosen to compare histologic evidence of mucosal injury (metaplasia) with immunochemical evidence of metaplasia. We will relate alterations in these markers to compare asbestos workers to subjects with a variety of other lung diseases and to normal control subjects. We hypothesize that:

1. The attack rate of metaplasia should be very high in asbestos workers.
2. That smoking history should correlate directly and retinoid ingestion inversely with evidence of metaplasia.
3. That smoking cessation may be important in modulating these markers.

Methodology

Fourteen asbestos workers received one bronchoscopy each. Twelve subjects were Connecticut residents, while one each traveled from Florida and Virginia at their own expense to participate. The workers included eight smokers and six subjects who had discontinued smoking three months to five years prior to the bronchoscopy. The average age was 57 years (range 43-70). All are or were insulators with over 20 years exposure to asbestos. The bronchoscopic procedure was smooth. One minor complication was noted. An episode of presyncope which was controlled by terminating the procedure prematurely. We changed the protocol to eliminate codeine premedication. We have had no recurrence. Additionally, we have studied four subjects with mild COPD, nine subjects with bronchiolitis obliterans and seven subjects with IgA deficiency and recurrent lung infections.

Each patient received an occupational history, dietary history, social history (tobacco and alcohol use). Pulmonary functions tests and chest x-ray provided clinical evidence of asbestos- or smoking-induced lung disease. Nasal wash and parotoid fluid were obtained and a bronchoscopy was performed. During the bronchoscopy, I obtained four to six biopsies from subcarinas of large airways. Bronchial brushings were suspended in saline. Finally, a lavage was obtained from an unbiopsied airway.

Progress and Accomplishments

Pulmonary functions tests of asbestos workers showed a restrictive ventilatory defect. Total lung capacity was reduced to 75% of predicted and diffusion capacity was decreased to 77%. Chest x-rays revealed pleural plaques in all subjects and parenchymal fibrosis in 4 of 14 workers.

Bronchial biopsies disclosed metaplasia in seven of eight subjects. Tissue is being processed for the remaining 6 patients. We plan to stain these biopsies to study the distribution of kertins immunohistochemically. Furthermore, we have performed in situ hydridization of c-DNA probes for keratins (provided by Dr. Elaine Fuchs) and for c-ras and c-myc oncogenes. Grain counts of these preparations are in progress. Bronchial brush cells (79% epithelial cells) will be lysed, cytosol proteins will be recovered and eletrophesed into polyacrylamide and western blots will be stained for keratins.

Bronchial lavage cells contained 90% alveolar macrophages, 8% lymphocytes and 2% neutrophillic polymorphonuclear leukocytes.

Bronchial lavage fluid from workers (n = 12) has shown detectible keratins in 75%. This contrasts with the frequency of keratins in other diseases: none of 4 COPD patients; none of 7 with acute infection and three of 9 with bronchiolitis obliterans (chronic bronchial inflammation).

Significance

We have noted a high frequency of metaplasia in this population of asbestos workers who are current or former smokers. Although we are unable to say the best way to assess this lesion (histologic or biochemical analyses), we are confident that this group represents an excellent target population for attempts to modify pharmacologically epithelial growth and differentiation. In the coming years, we will collect more patients, assess the data to evaluate the utility of various markers (biopsy, lavage, saliva, nasal wash) and recruit some of these patients with metaplasia for an intervention study.

Tannin as a Byssinotic Agent-effect on Endothelial Cells

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Occupational Lung Diseases
5 R01 OH02116-03
06/01/85 - 05/31/88
$63,709 ($178,383 Cum)

Objectives

Byssinosis is a significant occupational health problem among cotton mill workers. The source of the byssinotic agent appears to be the cotton bract, although the specific causative bract component or components are unknown. This project comprises an evaluation of the effect of condensed tannin, a purified component of the cotton bract, on vascular endothelial cells. The overall goal of this proposal is to characterize the effects on vascular endothelial cells medicated both by purified tannin and by aqueous extracts of the cotton bracts from which the tannin was isolated. We believe that an understanding of the cellular toxicity of tannin may offer insights into the pathophysiology of byssinosis.

Methodology

Specific Aim 1: Characterization of time and dose dependencies of endothelial cellular toxicity mediated by tannin and bracts extracts.

The experiments addressing this specific aim consist of assays of cellular toxicity using ^{51}Cr release from endothelial cells exposed to varying doses of tannin for varying periods of time. These experiments yield toxicity curves of tannin effects on the endothelial cells.

Specific Aim 2: Determination if tannin alters paracellular endothelial permeability prior to the onset of cell death.

Experiments in this specific aim are intended to determine if the barrier function of endothelial cells is affected by tannin, that is, if an endothelial monolayer might be affected by tannin in other ways than simple cell death. The methods to accomplish this aim involve measurement of paracellular permeability using exclusion of vital dye by endothelial cell monolayers.

Specific Aim 3: Determination if tannin promotes detectable changes in endothelial cell structure or function prior to cell death.

The experiments in this specific aim will determine if significant sublethal injury is induced in the endothelial cells by tannin, and whether such injury significantly affects cellular function. These experiments assess several parameters of cellular function, including cellular protein synthesis, serotonin uptake, and pinocytotic rate.

Specific Aim 4: Characterization of the effects of chronic, sublethal exposure to tannin on endothelial cell function.

This last specific aim builds upon the data accumulated in the first three specific aims. We will determine the minimum tannin dose and extent of exposure required to induce the various specific toxic effects delineated in the first three specific aims. This is of major importance in regard to cumulative toxicity that a cotton mill worker could experience over a many year exposure to cotton bracts. An important aspect of these experiments is the reversibility of any of the observed tannin-induced effects described above.

Progress and Accomplishments

Specific Aim 1: The experiments proposed in Specific Aim 1 have been accomplished and are described in the publication listed below. We have defined the time and dose dependency of tannin toxicity to endothelial cells, and compared these data to tannin effects on peripheral skin fibroblasts. These data show that the endothelial cell appears to be particularly, perhaps uniquely, susceptible to tannin-mediated effects.

Specific Aim 2: We have not as yet performed experiments that address paracellular endothelial permeability, although we have now succeeded in consistently growing the endothelial cells on the cytodex beads. Therefore we expect that these experiments of Evan's blue dye inclusion can be accomplished in the next six to nine months.

Specific Aim 3: We have performed experiments of cellular protein synthesis during the initial, prelethal, period of exposure to tannin as described in the grant proposal. Although cellular release of ^{51}Cr does not occur until after three hours of tannin exposure and protein synthesis continues for several hours following the initiation of a toxic tannin dose, synthesis of all proteins is profoundly diminished within the first half hour of exposure. We have determined this using endogenous labeling experiments of serial pulses of ^{35}S-methionine in tannin exposed cells. We are presently performing experiments of cellular transport of various amino acids to determine if this toxicity is mediated by, among other things, an interference with transmembrane uptake of amino acids and glucose. Our morphologic observations accomplished in Specific Aim 1 lead us to believe that the primary toxic effect of tannin is on cellular membrane function.

Significance

We believe that the major significance of the project so far is in describing the dose and time dependencies of endothelial cellular toxicity to cotton bract tannin. We find it of great interest that skin fibroblasts, a mesenchymal cell remote from the vascular tree, is relatively resistant to tannin-medicated toxicity. Further, there are obviously profound membrane effects provoked in the endothelial cells by cotton bract tannin. In addition, we have shown that the toxicity to endothelial cells by crude cotton bract extract is primarily accounted for by the tannin in the extract. Since bract extract is a complicated mixture of materials, this observation supports our original hypothesis that tannin may be an important byssinotic agent.

Publications

Johnson CM, Hanson MN, Rohrbach MS: Toxicity to Endothelial Cells Mediated by Cotton Bract Tannin: Potential Contribution to the Pathogenesis of Byssinois. Amer J of Pathology 122:399-409, March 1986

Johnson CM, Hanson MN, Rohrbach MS: Endothelial Cell Cytotoxicity of Cotton Bracts Tannin and Aqueous Cotton Bracts Extract: Tannin is the Predominant Cytotoxin Present in Aqueous Cotton Bracts Extract, Envir Health Perspectives 66:97-104, 1986

Polonium-210 Radiation and Dithiols

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Occupational Lung Diseases
5 R01 OH02185-02
01/01/86 - 03/31/88
$71,369 ($146,501 Cum)

Objectives

Polonium-210 (Po^{210}) is a alpha emitting radioactive daughter of 222 radon. For man, inhalation and ingestion are the major portals of entry. There have been "disasters" caused by accidents with Po^{210}, e.g. the Windscale accident in England (7b). One of the occupational dangers of uranium miners is Po^{210} inhalation. There has been recent concern about radon and Po^{210} concentrations in the interior air of poorly ventilated buildings and homes resulting from materials used in site preparation and/or construction materials. In addition, accidents in nuclear facilities have occurred with Po^{210}.

The objectives of the present study are to find antidotes and decorporations agents effective against Po^{210}.

Methodology

Male rats were injected with Po^{210} and various potential decorporating agents (or antidotes) were injected beginning one hour later. Animals when survival was studied, were followed for about 120 days. When decorporating studies were done, rats were autopsied 21 days after the polonium injection and the tissue content of Po^{210} determined.

Progress and Accomplishments

Po^{210} can bind thiols and thiol-containing proteins *in vivo*. Since thiol-containing chelating agents compete with many thiols for heavy metals, a number of the chelating agents have been investigated as protective agents against the lathal effects of Po^{210} and as tissue decorporating agents for it. Rats given Po^{210} (40 μCi/kg) ip had a median survival time (mst) of 39 days. The mst was increased to 106 days when N-(2,3-dimercaptopropyl)pthalamidic acid (DMPA), meso-dimercaptosuccinic acid (DMSA) or the Na salt of 2,3-dimercapto-l-propanesulfonic acid (DMPS) was administered sc ($p<.002$). Decorporation studies were performed by giving rates Po^{210} (0.4μCi) sc, followed by a series of thiol injections beginning one hour later. After 21 days, kidney levels of Po^{210} in rats given DMPA were only 28% of those of the untreated controls and significantly lower than those receiving DMSA, DMPS, N-acetyl-L-cysteine, or WR2721. After DMPA treatment, the Po^{210} levels of the spleen were 25% of the saline-treated control. DMPA appears to be a new and consistent decorporating agent for Po^{210}.

Significance

Po^{210} has been used as a source of thermal power in earth satellites, a neutron-productіng initiator, an antistatic device and in weapon production. At least two Po^{210} accidents in a nuclear working environment have been reported in the literature, one in 1975 and the other in 1983. The results of the present experiments with DMPA may be of help in the treatment of humans involved in such accidents. Certainly, for emergency treatment of accidental situations, the finding that treatment can be delayed for at least one hour is encouraging. It is planned to extend these experiments in order to determine how long after exposure to Po^{210} the DMPA will be useful and effective as a decorporating agent.

Publications

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Mechanism and Treatment of Phosgene Poisoning

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Occupational Lung Diseases
5 R01 OH02264-02
08/01/84 - 12/31/87
$126,860 ($340,778 Cum)

Objectives

The purpose of this research was to develop an effective and practical therapy for acute toxic gas inhalation.

Methodology

Lungs from male New Zealand white rabbits (3-4 kg) exposed to 2000 ppm·min phosgene were perfused in a recirculating manner with modified Krebs buffer. Vascular and tracheal pressures, and lung weight gain from edema formation were monitored as indices of lung injury.

Progress and Accomplishments

In the NIOSH annual report for 1986, we reported that agents elevating cyclic AMP given after phosgene exposure markedly attenuated lung injury 4 hours later, whereas methylprednisolone post-treatment did not prevent lung injury. Lungs from unexposed control rabbits or rabbits exposed to 2000 ppm-min phosgene and not treated gained $.08 \pm .01$ and $1.20 \pm .39$ gm/min, respectively. In contrast, lungs from rabbits treated with the beta-2 agonist terbutaline (20 ug/kg SQ, then 10 ug/kg/hr) or the phosphodiesterase inhibitor aminophylline (16 mg/kg IV, then 8 mg/kg IP every 2 hours) gained $.21 \pm .08$ and $.24 \pm .01$ gm/min, respectively ($p < .05$ compared to phosgene-exposed untreated lungs). To determine whether the protective effect of agents elevating cyclic AMP was from an effect on lung alveolar-capillary membrane permeability, or simply an effect of small decreases in inflow pulmonary artery pressure, we studied an additional group of rabbits pretreated with agents elevating cyclic AMP before phosgene exposure. These lungs were perfused within 60 minutes of exposure. To study effects of these agents on alveolar capillary membrane permeability, we measured rate of lung weight gain at different left atrial pressures at the end of lung perfusion. Since pulmonary microvascular pressure (Pmv) is defined by $Pmv = Pla + 0.4\,(Ppa\text{-}Pla)$, where Pla is left atrial pressure and Ppa is pulmonary artery pressure, rate of fluid flux across the alveolar-capillary membrane is primarily dependent on membrane permeability and Pla at higher left atrial pressures when Ppa contributes much less to Pmv.

The findings from our research are as follows: 1. Untreated phosgene exposed lungs experienced marked weight gain from edema formation, whereas lungs treated with the beta agonists terbutaline and isoproterenol, the cyclic AMP analog dibutyrl cyclic AMP or the phosphodiesterase inhibitor aminophylline gained weight at rates similar to uninjured control lungs, and 2. The rate of lung weight gain increases markedly in phosgene exposed untreated lungs as left atrial pressure is increased. In contrast, lungs treated with beta-agonists, dibutyryl cyclic AMP or aminophylline gain weight at rates similar to uninjured controls. These results indicate that agents increasing cyclic AMP attenuate the increase in lung alveolar capillary membrane permeability occurring after phosgene exposure.

Significance

While the mechanisms of their protective effects is still under investigation, drugs used to treat asthma by elevating cyclic AMP may provide a practical and effective therapy for victims of workplace toxic gas inhalation. We recommend that our results be confirmed in other animal species before application to man.

Effect of Particle Load on Alveolar Clearance

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Occupational Lung Diseases
1 R01 OH02332-01
01/01/87 - 12/31/89
$128,448 ($128,448 Cum)

Summary

Airborne particles of an infectious, industrial or environmental nature are constantly inhaled and must be cleared by lung defense mechanisms. Several mechanisms of clearance in man are suspected, usually from small animal experiments, and these may vary with species, the composition and size of particle, site of its deposition, presence of lung disease and particle load. The purpose of this study is to examine the routes of particle clearance from deposition sites distal to the mucociliary escalator (long-term or "alveolar" clearance) in an effort to quantify the lung clearance mechanisms under normal conditions in an animal model close anatomically and physiologically to man. For this purpose, we will use the sheep because of the resemblance of its lung in size and structure to man and because its size and docility are consistent with highly desirable research methodology. Our approach will permit us to determine, in a quantitative manner, the routes of clearance of particles of 2 sizes (0.5 and 3 CMD) after deposition in the "alveolar" portion of the lung. The methods of assessing routes of particle clearance include 1) gamma camera imaging (for overall pattern and rate of clearance) in-vivo, and, in-vitro, after sacrifice and extirpation of the lung, 2) surveillance of routes of exist, such as a) whole lung lavage for the content of macrophage-related and unrelated particles, b) creation of tracheal stomae for collection and analysis of mucus for its particles and macrophages, c) lymph nodes for their radioactive content, reflecting particles, d) feces for total radioactive count reflecting particles eliminated through the airway, and 3) light microscopic corroboration of original sites of deposition and translocation during the course of clearance from extirpated lung tissue. In the process we should be able to quantify the portion of particles leaving alveoli by the tracheobronchial route (and the role of the macrophage therein), that portion clearing through the interstitium, and, of these, the fraction clearing through lymph or lymph nodes, and, finally, of the portion sequestered in the lung. We also shall determine how excess particle loads affect these avenues of clearance when given by inhalation as acute and chronic burdens of one to several orders of magnitude greater than control. This study should thus reveal potential factors which lead to failure of the clearance system responsible for eliminating respired airborne particles from the alveolar surface of the lung and which may consequently aggravate their inflammatory and fibrogenic potential in the lung parenchyma.

Lung Collagen in Silicosis: Fibrosis Mechanisms

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Occupational Lung Diseases
5 K01 OH00002-03
09/28/84 - 08/31/87
$32,400 ($97,200 Cum)

Objectives

There are four specific aims to be accomplished in this project:

1. To test the hypothesis that collagen crosslinking in silicotic rat lungs differs from that found in normal lungs in one or more of the following aspects: types and amount of crosslinks per collagen molecule, time course of crosslink formation, turnover of crosslinks, reducibility of crosslinks, and location of crosslinks.
2. To test the hypothesis that crosslinking in silicotic rat lungs differs from that found in bleomycin-exposed rat lungs.
3. To determine whether elastin crosslinking can be measured in the lungs of either normal adult rats or rats exposed to fibrogenic stimuli (silica, bleomycin), and if so, to determine if there are differences among the groups.
4. To develop simple screening assays of urine or blood, based on data obtained in Specific Aims #1-3, indicative of early silicotic changes.

Methodology

We have developed techniques for separation and quantification of both conventional amino acids and collagen crosslinks using a flexible reverse-phase HPLC system. The equipment, reagents, and elution programs used for such analyses have been described by us in detail (Reiser and Last, Liquid Chromatography 1:498-502, 1983).

Silicosis was induced in rats by intratracheal instillation of 50 mg of crystalline silica. Fibrosis was also induced by intratracheal instillation of 1-1.5 U of bleomycin. *In vivo* labelling of crosslinks was accomplished by implanting Alzet minipumps containing 800 μCi (^{3}H) lysine.

Progress and Accomplishments

During the third year of the project, *in vivo* labelled tissue was analyzed. Although our pilot studies indicated that lung tissue could be directly analyzed without prior fractionation, label incorporation was lower than we had anticipated. The procedure for analysis was therefore modified. Whole lungs were washed and digested with collagenase and the supernate was chromatographed on a Bio-Gel P-2 column, using a pyridine-acetate buffer, to separate the crosslinks from the bulk of the amino acids. The appropriate fractions were pooled and chromatographed by HPLC. Our data showed that a increase in labelled hydroxypyridinium (OHP) was not observed 1 and 2 months after minipump installation; however, 6 months after installation labelled OHP content was significantly higher in the silicotic lungs. These data are consistent with our findings on unlabelled tissue, and confirms that the time course of OHP synthesis *in vivo* is relatively long compared to that of the dysfunctional crosslinks.

In Specific aim 3 we proposed to examine elastin crosslinking in control and fibrotic rat lungs. We found no differences in lung desmosine (an elastin specific crosslink).

The 4th Specific Aim was the development of assays for measuring crosslinks excreted in urine to determine if changes in the lungs were reflected in the excretion of collagen breakdown products. Our initial protocol included precipitation with acetone. However, we found that a large proportion of the major collagenous crosslinks were present in the supernate, apparently bound to small peptides. In our revised protocol, we reduced an unfractionated aliquot with NaB^3H_4, hydrolyzed, and treated it with charcoal. We found no consistent differences in crosslink excretion patterns between control and silicotic rats.

Significance

In previous studies we have observed that lung collagen appears more "normal" metabolically in silicosis than in other types of fibrosis. In order to investigate the metabolic derangements that cause this apparently "normal" collagen to become deposited in fibrotic nodules, we decided to analyze collagen crosslinks as a way of tracking the metabolic fate of lung collagen from tropo-collagen to mature collagen fibers. Our data suggest that site-specific alterations in the level of lysine hydroxylation in silicosis, leading to observable increases in DHLNL and OHP, may be a key event in determining whether collagen is destined to become fibrotic. Identification of the derangement at the molecular level should provide insight into the still poorly understood mechanisms of this disease, and may lead to the development of rational strategies for both intervention and early diagnosis.

Publications

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Oxygen Free Radical in Pulmonary Fibrosis

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Occupational Lung Diseases
5 K01 OH00007-03
09/28/84 - 08/31/87
$32,400 ($97,200 Cum)

Summary

Exposure to asbestos and silica is associated with a variety of pulmonary disorders including pulmonary interstitial fibrosis. Although asbestosis and silicosis have been recognized as occupational lung diseases for nearly a century, the mechanism(s) by which these fibrogenic minerals induce lung cell injury and fibrosis is speculative, particularly at the cellular level.

In this proposal we hypothesize that oxygen free radicals may be one important mediator of lung cell damage and/or fibrosis *in vitro* and *in vivo* after exposure to fibrogenic minerals. To test this hypothesis, we will undertake the following studies:

1. Cytotoxicity induced by exposure *in vitro* to various concentrations of asbestos fibers and silicon dioxides will be determined in lung fibroblasts, alveolar macrophages and epithelial cells.
2. Production of superoxide radical (O_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH) by these cells after exposure to minerals at various concentrations will be monitored and correlated with results of #1 above.
3. Levels of superoxide dismutase (SOD) in lung fibroblasts, alveolar macrophages and epithelial cells after exposure to minerals *in vitro* and in lung sections of rats after inhalation of asbestos *in vivo* will be measured using quantitative biochemical assays and immunocytochemical techniques.
4. We will determine whether mineral-induced injury to alveolar macrophages, fibroblasts and epithelial cells *in vitro* can be prevented by the addition of scavengers of O_2 and OH.

These studies will be important in elucidating the importance of oxygen free radicals in mineral-induced lung cell injury, asbestosis and silicosis. In addition, they will contribute information on possible preventative and therapeutic approaches to these serious occupational diseases.

Silicosis: Immunological Abnormalities

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Occupational Lung Diseases
5 K01 OH00018-03
09/28/84 - 08/31/87
$34,128 ($98,928 Cum)

Objectives

To compare immunologic findings in silicotic subjects, identified in an epidemiologic study of respiratory disease among Leadville, Colorado residents, with those in community controls and in nonsilicotic miners having substantial past silica exposure.

Methodology

Silicotics and two appropriate comparison subjects matched for age within five years were interviewed by a local public health nurse; gave sputum samples for mycobacterial culture, and gave a blood specimen for complete blood and differential counts, immunoglobulins, immune complexes, autoantibodies, and separation of mononuclear cells. Detailed immunologic studies on peripheral blood mononuclear cells include lymphocyte proliferation to three mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and to two antigens (purified protein derivative, candida); helper cells suppressor cells, and natural killer cell phenotypes; lymphokine production, assayed for interleukin-2 and interferon; and evaluation of natural killer cell activity. Immunologic differences found for silicotic subjects in comparison to dust exposed and unexposed controls will be related to profusion of small and large opacities on chest radiographs and to the presence of mycobacterial infection due to M. tuberculosis or atypical mycobacteria. Silicotics wishing to have bronchoalveolar lavage in an independent study had constituents of lavage fluid compared to T-cell phenotypes in peripheral blood. Nonhispanic caucasian silicotics had histocompatibility antigen testing performed in a related study.

Progress and Accomplishments

The age-matched design was dropped since silicotic subjects, nonsilicotic miners, and community controls had comparable age ranges. We identified 61 silicotics, 66 nonsilicotics, and 34 community controls in our 161 subjects. Silicotics were defined as persons with a history of work in hardrock mining environments who had a profusion of small opacities of 1/0 or greater in the ILO classification. Only one community control was identified as having an abnormal chest radiograph.

Field data collection is complete. Laboratory data generation is nearing completion, with macrophage and polymorphonuclear phagocytosis slides, lymphokine assays, and T-cell subsets still being performed. All available data have been double-entered for accuracy purposes into SAS files. Statistical analysis is in progress. One manuscript has been submitted for publication concerning histocompatibility testing results in the subset of 49 nonhispanic silicotics. We found that silicotics had excesses of A29 and B44, which were statistically robust in the setting of multiple comparisons. No clinically important parameters correlated with these antigen excesses, although A29-positive silicotics had more immunologic abnormalities.

Five subjects had sputum cultures positive for atypical mycobacteria. Four of the subjects were silicotic (6.6%). Mycobacteria species included M. fortuitum, M. avium, M. gordonae, M. terrae complex, and M. chelonae. None of the subjects appeared to have clinically-significant infection.

Significance

We seek to identify immunologic factors which may predispose dust-exposed individuals to silicosis and which may predipose silicotic subjects to mycobacterial infections. Further understanding of the immunology of silicosis may suggest primary and secondary prevention measures which can be confirmed in future prospective studies. Our finding of histocompatibility antigen excesses in silicotics may be useful in screening for susceptible individuals, if substantiated in other population-based work.

Effect of Mineral Exposure on Macrophage Function

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Occupational Lung Diseases
5 K01 OH00019-03
09/28/84 - 12/31/87
$27,235 ($86,972 Cum)

Objectives

The effects of *in vitro* and *in vivo* (inhalation) exposure to minerals found in association with coal mine dust on function of individual alveolar macrophages will be studied. Comparison between *in vitro* and *in vivo* measurements will permit evaluation of the feasibility of using these *in vitro* techniques to predict *in vivo* effects.

Methodology

Dose-response and particle size dependent studies on functional alterations will be conducted using exposure levels which are sublethal to the cells. Two separate techniques will be used to evaluate toxic effects on cells lavaged from male Long Evans hooded rats. Electrophysiological voltage clamp experiments will be used to determine the effect of exposure on plasma membrane permeability to ions and electro-optical techniques will be used to measure the effect on production of superoxide anion, an antibacterial agent produced by the cell.

Progress and Accomplishments

An electro-optical method has been developed to measure superoxide (SO) release from single pulmonary alveolar macrophages (PAM) collected from lungs of rat by tracheal lavage. During adherence to the plate surface, cells released SO which was measured by the reduction of nitroblue tetrazolium (NBT), contained in the medium, to a formazan percipitate. Cells contained in a microscopic field were transilluminated at 550 nm (absorbance peak for formazan), televised and video recorded for forty minutes. The cells became progressively darker as the formazan percipitate was produced. Temporal changes in light intensity over the cells was measured by playing the recorded video images through a photometric analyzer. The light intensity data were converted to a relative mass of formazan produced using the Beer-Lambert law.

Heterogeneity was apparent since under the same conditions different cells released different amounts of SO at different rates. Several blockers of SO release were tested to determine the specificity of NBT reduction for SO release. Production of and rate of release of SO were reduced to 46% to 76% demonstrating that the majority of formazan production is due to SO release. The method developed is the first to quantify SO release from single cells and should be useful for studying single cell dynamics, heterogeneity and activation of superoxide producing system.

The effects of *in vitro* and *in vivo* exposure to minerals on macrophage ability to produce SO were examined. *In vitro* exposure to 0.025 or 0.05 mg/ml kaolin for 40 min resulted in no significant differences in either total formazan produced or the rate of formazan production. However, *in vitro* exposure to 0.025 mg/ml of quartz significantly reduced both the maximum and the rate of formazan production. This effect was blocked if the silica particles were first coated with a surfactant prior to exposing the cells. *In vitro* exposure to coal mine dusts in the same concentration as silica did not affect either the maximum or the rate of production of formazan. In contrast to the *in vitro* results, PAM lavaged from animals that had inhaled quartz dusts for 2 or 4 weeks exhibited increased formazan production. PAM analyzed from animals sacrificed 3 days after the end of exposure showed significant increases in production. This increase in average cellular response was due to the appearance of a high responding group of macrophages. This elevation was decreased in cells from animals sacrificed 10 days after exposure and cells analyzed 31 days post-exposure were not significantly different from controls. These results might suggest that there is an initial acute response to exposure of quartz that decreases the ability of PAM to produce SO, followed by recruitment or production of PAM with increased SO producing capability. It appears that removal of animals from the source of exposure permits PAM to return to control levels of SO production.

Measurements have been made in the electrophysiological portion of the study using whole cell patch clamping techniques. The effects of ionic current blockers have been used to determine control responses of rat alveolar macrophage plasma membranes. Macrophages demonstrated both outward and inward potassium currents. Appearance of these currents depended on the time after the cells had been removed from the animal and placed in culture. Preliminary simultaneous measurement of superoxide production and plasma membrane electrical changes following stimulation demonstrated that stimulation appears to block the outward potassium current. These data and the effects of mineral exposure on the currents are still being analyzed.

Significance

Comparison between *in vivo* and *in vitro* exposure will permit evaluation of the feasibility of using these *in vitro* techniques to predict *in vivo* effects. This interdisciplinary approach should yield important information on the cellular sites of toxicity of minerals and should provide data to aid in assessing risks following inhalation.

Publications

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Gas and Particle Deposition in Tracheobronchial Airways

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Occupational Lung Diseases
5 K01 OH00022-03
09/28/84 - 08/31/87
$32,400 ($97,200 Cum)

Objectives

The research is the first stage of a program focused on the development and critical evaluation of quantitative predictive models for gas and aerosol deposition efficiency in lung airways in humans and animals under a variety of respiratory modes and rates. The initial objective is to develop realistic physical models of the tracheobronchial airways of humans and experimental animals. The second objective is to use the models to examine the intrabronchial dose distribution of both gases and particles. The ultimate objective is to develop predictive models for deposition using both theory and experimentally derived coefficients, including models that account for the pronounced nonuniformity of epithelial deposition on the airways.

Methodology

Hollow airway casts of human and dog tracheobronchial airways were built. The airways were cast with microcrystalline petroleum wax to the level of the terminal bronchioles. Morphometric measurements were made on the wax casts through the fifth generation for all branches, and to the terminal airways along three paths of our "single pathway" models. All branches secondary to the three primary paths were also measured. Hollow casts were produced by coating the solid wax casts with silicone rubber. Flow distribution measurements were then carried out. The pressure drop through the cast was less than 10 Pa (1 mm H_2O), so a pressure balancing system was devised in which the pressure balance between the sections could be maintained. Pulsatile flow was obtained by using a positive pressure respirator (Monaghan 170C) with a switching valve (Rudolph 1400) to simulate inspiratory flow cycles. Measurements of the flow distribution under pulsatile flow were compared with that for constant inspiratory flow for equivalent mean inspiratory flow rates in the trachea. The mean inspiratory flow rate was determined from the measured integrated flow volume and duration of a pulse.

Progress and Accomplishments

Two hollow airway casts were produced, one human and one canine. There were over one thousand terminal airways on each hollow cast. The distribution of airflow was measured at constant flow rates of 15, 30, 45 and 60 L min^{-1} for the human, and 7.5, 15, 22.5 and 30 L min^{-1} for the canine. These are equivalent to minute volumes of 6, 11, 17 and 22 L min^{-1} for the human and 3, 6, 8 and 11 L min^{-1} for the canine assuming a breathing pattern of 3/8 inspiration, 3/8 expiration with pauses of 1/8 of a cycle after inspiration and expiration. The measured flow rates in a given airway segment for different flow rates into the trachea were fitted by linear regression to an equation of the form $y = ax^b$ where, y = the flow rate in the airway segment, x = the tracheal flow rate, and a and b are fitted parameters. When 'b' is near unity, 'a' represents the fraction of the total flow which passes through the segment. The coefficient of determination (r^2) was calculated for each regression of four (or more) different flow rates. Measured flow values can be combined for appropriate bifurcating networks to obtain parameters for particular lung segments, single lobes, or for each lung. For the complete pairs of lungs, the value of b is 0.991 for the canine and 1.006 for the human. The value of b is less than 1 for most airways in the upper lobes and greater than 1 for airways in lower lobes. This indicates a redistribution of flow from the upper to the lower lobes as the flow rate increases. The difference is more striking in the canine lung where the pattern is more consistent. The pattern is similar for pulsatile flow, which also further increases the deviation from linearity for a given airway.

Significance

The proper development of dose-response relationships for occupational respiratory diseases requires understanding of the determinants of the dose to critical respiratory tract tissues from inhaled gases and aerosols. Knowledge of the deposition patterns and efficiencies within airways is essential for the production of reliable dosimetric models. Predictions of aerosol deposition in the central airways based on uniform deposition are clearly unsatisfactory. Present models are not able to deal with the inhomogeneity of deposition demonstrated in the cast system. It is anticipated that as this work progresses, more realistic theoretical models will result from better understanding of the airflow patterns in the airways. In addition the differences and similarities between deposition patterns in the human dichotomous branching tracheobronchial tree and the canine monopodial branching will be documented. This is important if results obtained in experimental animal models are to be properly extrapolated to humans.

Publications

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Macrophage, Particulate, and Carcinogen Metabolism

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Occupational Lung Diseases
1 K01 OH00065-01
07/01/87 - 06/30/90
$32,389 ($32,389 Cum)

Summary

Particulate ferric oxide (Fe_20_3) is known to have an enhancing effect on benzo (a) pyrene (BaP)-induced lung carcinogenesis and although the mechanism of this cocarcinogenic action remains unknown, several investigations have implicated altered BaP metabolism. An important biological response to inhaled particulates is inflammation and the subsequent ingestion of particulate by alveolar macrophages (AM). The activation of macrophage arachidonic acid metabolism is an important component in the inflammatory response and aspects of this metabolism have been associated with metabolic activation of BaP. The long-term objective of this research is therefore to determine the role of inflammation, specifically phagocytosis-induced arachidonic acid metabolism in AM in the potentiation of BaP metabolism by particulate

Using AM isolated from male Syrian Hamsters, the specific aims are to determine: (1) The relationship of arachidonic acid metabolism induced by phagocytosis of Fe_20_3 to altered BaP metabolism induced by the same particulate, (2) the ability of aluminum oxide, a particulate that does not enhance BaP carcinogenicity, to mimic the actions of Fe_20_3, (3) the effect of pretreatment with BaP on agonist induced arachidonate metabolism, and (4) the effect of phagocytic and nonphagocytic activation of arachidonate metabolism on the metabolism of BaP in cells preloaded with BaP.

These specific aims will be addressed in studies utilizing radiolabeled arachidonic acid and BaP. Metabolite of these two biochemicals will be analyzed in incubation buffer and cells following exposures to various combinations of BaP, particulate and selective inhibitors of arachidonic acid metabolism. Analyses will be performed using extraction procedures (BaP and arachidonic acid), HPLC (BaP and arachidonic acid) and neutral alumina column chromatography (BaP) for separation of metabolites.

In the evaluation of occupational hazards that may lead to increased susceptibility to lung cancer, the cocarcinogenic potential of an exposure may become an important consideration. The mechanisms of action of cocarcinogens are, however, still poorly understood. This research will provide useful information on the involvement of a critical component of the inflammatory response, arachidonic acid metabolism in the AM, in particulate modified BaP metabolism.

Inhaled Toxic Agents: an Evaluation of Dose

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Occupational Lung Diseases
1 K01 OH00067-01
07/01/87 - 6/30/90
$32,400 ($32,400 Cum)

Summary

Numerous chemicals found in the workplace have been demonstrated to induce cancer in animals. The principal route of entry of many of these chemicals is the respiratory system, a highly efficient heat and mass exchanger. In assessing possible regulatory controls or a proposed standards for these chemicals, the question arises as to the relevance of the animal exposure data to infer human health risk. To address this question, two major toxicological factors must be considered. These factors, which may vary among species, are: (1) The target tissue dose for any specified exposure to a toxic agent (mass delivered/unit area), and (2) The biological response as a function of a specified target tissue. In this study, we will focus on fundamental questions regarding the quantitative evaluation of target tissue dose within the respiratory system. A mathematical model will be developed to predict regional respiratory dose as a function of inhaled concentration and breathing rate. The model developed from a first principles approach, will be applicable to humans and laboratory animals provided the model parameters are available for each species. Fundamental to assessing the radial transport of the gas to the airway surface is the mass transport coefficient. We have evaluated this parameter for restful nasal breathing in humans. In this study, we propose to further evaluate this parameter for both oral and nasal breathing over a wide range of flow rates. An initial study to evaluate the absorption rate of gas in the upper airway using these coefficients is reviewed in the preliminary study section and is compared to *in vivo* measurements. We intend to develop the model to predict the absorption of several gases and include the possible reactions between them. As such the model will be the first to consider the additivity, synergism, or potentiation of dose in gas mixtures. Additionally, because submicron particles are transported principally by diffusion (39) as opposed to impaction or gravitational settling, we will assess the feasibility of utilizing the convective transport coefficients and the model to describe deposition of submicron particles by comparing model predictions and *in vivo* data.

Occupational Health Factors in Farmer's Lung Disease

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Occupational Lung Diseases
5 RO3 OH02098-02
04/01/85 - 07/31/87
$20,723 ($41,694 Cum)

Objectives

Farmer's Lung Disease (FLD) is a prototype hypersensitivity pneumonitis which may progress to interstitial lung fibrosis. This study was designed to examine the question of whether individuals with circulating antibodies to FLD antigens are at increased risk for developing the disease. Specific objectives include:

1. Assessment of associations between circulating antibody status, and the prevalence of chronic respiratory symptoms, the presence of impaired lung function and the incidence of FLD.
2. Assessment of occupational and environmental factors which affect circulating FLD antibody status.

Methodology

This study is a prospective epidemiologic analysis of a population-based random sample of 1444 farming individuals living in central Wisconsin. When tested in 1977, 143 subjects had circulating FLD antibodies (by the double immunodiffusion assay). These antibody positive individuals were matched by age, sex and smoking status to 143 antibody negative individuals. Spirometry tests and a questionnaire, which included the American Thoracic Society standardized questions on respiratory symptoms and smoking, were administered on all participating subjects. Statistical analyses were performed using SAS.

Progress and Accomplishments

Two hundred twenty-four individuals participated in the study and another 42 people responded to questions related to smoking status, current health, educational level and current occupational status (93% response). Statistical analyses of the edited data set have shown:

1. Chronic phlegm, chronic cough and dyspnea on exertion occurred significantly more frequently in the antibody positive cohort than the antibody negative cohort after controlling for the effects of smoking.
2. Usual cough, usual phlegm and wheezing occurred equally frequently in the antibody positive and negative cohorts.
3. Pulmonary impairment, as a function of antibody status, is not evident.
4. The pattern of respirator use was similar for both cohorts. Fifty-five percent of the positive cohort and 64% of the negative cohort claimed they never used any sort of respiratory protection during farm work.
5. Number of acute febrile episodes related to exposure to organic dust was predictive of chronic respiratory symptoms and of spirometric measures of small airways disease.

Significance

FLD antigens are commonly found in agricultural dust and approximately 10% of Wisconsin farmers have circulating antibodies to these antigens. Most individuals with FLD antibodies have never experienced a symptomatic acute FLD illness and even fewer develop chronic FLD. The significance of the presence of circulating antibodies in relation to the pathogenesis of FLD has been a point for discussion for a number of years. More recently, Organic Dust Toxic Syndrome (ODTS) has received a considerable amount of attention in the literature. ODTS is an acute inflammatory illness which follows exposure to high concentrations of dusts and molds, whereas acute FLD is an allergic disease which affects only sensitive individuals after minimal to moderate exposure. Formerly, the presence of circulating antibodies helped to distinguish FLD from ODTS; however, it is now accepted that ODTS patients may have antibodies to FLD antigens. Our results support the hypothesis that the presence of circulating antibodies function as an index of exposure to thermophilic actinomycetes and, more generally, to agricultural dust, but do not have prognostic significance.

Publications

Guernsey JR: The Prognostic Significance of Farmer's Lung Disease Antibodies Relative to Measures of Respiratory Disease in a Wisconsin Dairy Farming Population, Ph.D. Thesis, U of Iowa, December 1985

Guernsey JR, Morgan DP, Marx JJ, Horvath EP, Pierce WE, Merchant JA: Respiratory Disease Risk Relative to Farmer's Lung Disease Antibody Status, Proc International Symposium of Health and Safety in Agriculture, Saskatoon Saskatchewan, in press

Coal Workers' Respiratory Disease Program Evaluation

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Occupational Lung Diseases
1 R03 OH02189-01
12/01/85 - 11/30/87
$24,675 ($24,675 Cum)

Objectives

To determine if treatment in a pulmonary rehabilitation program (Pennsylvania Coal Workers' Respiratory Disease Program or CWRDP) reduces morbidity in Program participants (coal miners) as measured by hospital days from respiratory illness, and to determine if the benefit in dollars exceed the cost of the Program if the treatment is shown to decrease morbidity.

Specific Aims:

1. To select 55 treated white males and 55 non-treated white male miners selected from a cohort of 700 coal miners with initial Program enrollment in 1979.
   a. Treated - miners returning at least one time for evaluation and treatment after the 1970 initial enrollment.
   b. Non-treated - miners never returning following 1979 initial enrollment.
2. To compare differences in the treated and non-treated miners with regard to length, frequency, and diagnoses for hospital days from respiratory illness pre and post Program enrollment.
   a. Respiratory Hospital Days - any hospitalization with a primary or secondary diagnosis of respiratory disease (as categorized by the International Classification of disease or ICD codes).
   b. Pre enrollment - period from 1974 to 1979 date just prior to initial enrollment.
   c. Post enrollment - 1979 date of enrollment to 1983.
   d. Data for respiratory hospitalizations were obtained for 5 years pre and 5 years post Program enrollment in the 2 groups.
   e. Comparisons are being made for hospital differences between the 2 groups and for differences pre and post enrollment within each group.
3. To observe differences in the average annual ventilatory decline for miners returning for treatment as compared with long-term declines for respiratory patients, which have been cited in the literature.
4. To determine if hospitalization differences exist within and between treated and non-treated groups and to test the significance of these differences using analysis of variance.
5. To adjust for confounding variables such as smoking using multivariate analysis.
6. To calculate the benefit-cost analysis if data analyses demonstrate less respiratory hospital days in the treated group.

Methodology

This non-concurrent prospective study looks at a subset of miners from a cohort (numbering ~700) with initial CWRDP Program enrollment in 1979. Using clinic and hospital records, the treatment effects on respiratory hospitalizations (and, when available, pulmonary function and arterial blood gas) will be observed in treated and non--treated miners from the original cohort.

For the ~250 (out of ~700) miners returning for treatment, the pattern of ventilatory decline as measured by FEV_1 and FVC and by pO_2 and pCO_2 will be observed and compared to findings in the existing literature.

If the treated miners are found to have reduced morbidity as measured by respiratory hospital days, a cost-benefit analysis will be undertaken. The cost of medical and hospital care will be contrasted for the treated and non- treated groups and evaluated against the benefits (based upon the potential savings resulting from reduced hospitalization from respiratory illness).

Progress and Accomplishments

The hospital record review is essentially completed for the treated and non-treated groups. A few more records are in the process of being obtained. Coding of the hospital data is now being done and computer entry will soon follow. Analyses of the data will then ensue.

Early analyses of the 1979 enrollment data (which combined treated and non-treated miners) revealed a statistically significant 10% lower mean % predicted FEV_1 in persons with hospital stays exceeding 7 days. If the program objective of improving functional status is met, then a reduction in respiratory hospital days in the treated group would logically be expected to follow.

This study will demonstrate whether differences in respiratory hospitalizations are found in the 2 groups. Analyses will be done to determine the association of treatment to respiratory hospital days and whether presence or absence of treatment affects the respiratory hospital days negatively or positively.

If presence of treatment is associated with decrease in respiratory hospital days and absence of treatment associated with increase in respiratory hospital days, the strength of these associations will be tested statistically. If the associations are statistically significant (i.e. strong association), the argument for continuation of these programs would be greatly strengthened.

This study design may also become a model for evaluating other health programs for occupationally related diseases.

Very early preliminary analyses for this study have been presented at the International Conference on the Health of Miners sponsored by the National Institute of Occupational Safety and Health, World Health Organization, University of Pittsburgh, and the American Conference of Governmental Industrial Hygienists in Pittsburgh, Pennsylvania, on June 2-7, 1985. The presentation was recently published in the Annals of the American Conference of Governmental Industrial Hygienists for 1986.

Publications

Ashizawa AE: Coal Workers' Respiratory Disease Program Evaluation. In R.W. Wheeler (Ed.), Annals of the American Conference of Governmental Industrial Hygienists: Int'l Conference on the Health of Miners Vol 14, Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, 1986

Cotton Bract and Human Airway Hyperresponsiveness

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Occupational Lung Diseases
1 R03 OH02265-01
09/01/86 - 08/31/87
$23,000 ($23,000 Cum)

Objectives

The overall aim of this project was to determine if inhalation of cotton bract extract (CBE) alters non-specific airway responsiveness to methacholine.

Methodology

Healthy volunteers underwent a double-blind, random, crossover trial of inhalation of CBE or normal saline sham. Methacholine dose-response curves were established prior to each provocation and again two, eight, twenty-four and one hundred sixty-eight hours later. The primary response parameter was the dose of methacholine required to induce a 25% decrease in the expiratory flow at 60% of the vital capacity below total lung capacity on the partial flow-volume curve (MEF40%(P)).

Progress and Accomplishments

Five of the 13 individuals tested demonstrated a ventilatory response to CBE with a 20% or larger decrement in the MEF40%(P)); no individual demonstrated such change with NS aerosol sham. For the group, the peak decrement in MEF40%(P) was 76.5 ± 20.3% of baseline (mean ± SD) occurring approximately 60-90 minutes post-provocation while the peak decrement following normal saline was 88 ± 10.6% of baseline occurring immediately after inhalation.

Changes in airway responsiveness to MC were mild and transient. For example, the PD25MEF40%(P) for the group (mean ± SD) was 51.3 ± 41.1 mg/ml at 2, 8, 24 and 168 hours (7 days) respectively. Following a pre-sham baseline of 50.4 ± 43.2 mg/ml, PD25MEF40%(P) was 57.6 ± 83.8, 153.8 ± 148, 81.9 ± 106, and 64.0 ± 62.7. A repeated measures analysis of variance on the acute, same day changes (i.e., 2 and 8 hours post-provocation) resulted in a statistically significant effect of CBE on airway responsiveness with the log transformed data (F=4.83; P=0.048). However, an overall analysis of variance with all repeated measures (i.e., all 4 post-provocation time points) did not demonstrate a statistically significant CBE effect (F=2.73; P=0.124).

Significance

Recent evidence obtained from studies of human volunteers indicate that cotton bract extract is an inflammatory stimulus. Since current evidence suggests that airway inflammation is involved in the development of airway hyperactivity, this investigation examined the effect of cotton bract inhalation on non-specific airway reactivity to methacholine. Our results indicated a mild and transient increase in airway responsiveness following CBE. The potential public health importance of these relationships stems from the increasing evidence that airway hyperreactivity plays a role in the accelerated loss of lung function in patients with chronic obstructive lung disease. Specifically, if airway hyperreactivity is identified as an important link in the transition from acute, reversible disease to chronic debilitating byssinosis, intervention in the form of industrial hygiene policy or even pharmacologic management may be important in preventing this transition.

Publications

Witek TJ, Mazzara C, Zuskin E, Beck GJ, Buck MG, Schachter EN: Bronchial Responsiveness Following Inhalation of Cotton Bract Extract. Am Rev Respir Dis, in press

Witek TJ, Stack E, Schachter EN: The Consistency of Repeated Flow-Volume Maneuvers in Airway Challenge Studies. Respiration, 1988, in press

Immune Responsiveness in Chlorine Exposed Rats

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Occupational Lung Diseases
1 R03 OH02425-01
09/29/87 - 09/28/89
$22,500 ($22,500 Cum)

Summary

Survivors of an acute, sub-lethal irritant gas exposure may have persistent long-term respiratory symptoms and/or functional impairment. Symptoms may result from enhanced lung permeability with subsequent increased sensitivity to antigen. Inflammation is the hallmark of irritant gas injury and many of the superficial differences observed are related to the site of action, which is determined by physical chemical properties of the irritant, rather than intrinsic differences in the response. Chlorine is widely used and frequently involved in industrial or transportation accidents. It has intermediate solubility; thus, may exert its primary effect on all portions of the respiratory tract. Since purposeful exposure of humans to high levels of chlorine could never be justified, in this proposal, long-term respiratory effects following an acute chlorine exposure will be evaluated using an animal model.

This proposal is designed to test the hypothesis that acute chlorine exposure increases, in dose-dependent fashion, antigen sensitivity. It also hypothesized that these effects are long lasting rather than transient. To test this hypothesis, an animal model will be employed. BALB/c mice will be exposed to chlorine gas, in dose-dependent fashion, insuring induction of a range of sub-lethal injuries. Effects of chlorine exposure will be evaluated by histologic analyses of lung, trachea, major bronchi, kidney, adrenal, liver, and spleen tissue. It is anticipated that analysis of lung and airway tissue will provide the most important information. To evaluate alterations in populations of immunocompetent cells, monoclonal antibodies and flow cytometry will be used. Lymphoid cells from lungs, and for comparison a peripheral source (spleen), will be obtained from chlorine exposed and control animals. Cells will be obtained from lavage fluid, lung parenchyma, and spleen. Briefly, the pulmonary vasculature is flushed with 10 ml Hanks Balanced Salt Solution. The trachea is cannulated with polyethylene tubing attached to a 21-gauge needle and 3 ml syringe. Lungs are washed 5 times with media and fetal calf sera. Retrieved lavage fluid is pooled and centrifuged. Following lavage, lungs are minced and collagenase treated. Treated lung cells are counted and viabilities are determined by trypan blue exclusion. Spleens are dissociated into media. Phenotypic assessment of lymphoid populations are made utilizing commercially available monoclonal antibodies and flow cytometry. Cell populations to be analyzed are total T cells, T suppressor/cytotoxic cells, T helper/inducer cells, Ia bearing cells, surface immunoglobulin positive (B cells) cells, and natural killer cells. Following assessment of damage resulting from gas exposure, three chlorine concentrations will be selected for subsequent evaluation. In those studies, animals will be exposed to chlorine and sensitized (2 hours, 1, 7, 30, or 60 days post-gas exposure), to aerosolized ovalbumin (OA). Local and systemic antibody responses will be quantitated using an enzyme linked immunosorbent assay and passive cutaneous anaphylaxis testing. To evaluate cellular responsiveness, lymphocyte transformation, using cells isolated from the spleen and the lung associated lymph nodes, will be performed with OA or concanavalin A.

Experiments outlined in this proposal are designed to examine the outcome of chlorine exposure, but results will likely apply to other primary irritants by virtue of common pathogenic mechanisms and/or respiratory tract and lung inflammation. If the findings suggest enhanced susceptibility to specific sensitization, they will provide guidance in design of studies or surveillance programs for exposed workers.

International Conference on Biological Mechanisms

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Occupational Lung Diseases
1 R13 OH02117-01
02/01/86 - 01/31/87
$10,000 ($10,000 Cum)

Summary

The occupational lung diseases (e.g., the pneumoconioses--asbestosis, silicosis, coal workers' pneumoconiosis--and lung cancer, mesothelioma, and airways disease) constitute a major portion of disability among workers. NIH/NIOSH have labeled occupational lung disease number one among occupational diseases and, even though the epidemiology of these disorders has been well described over the past two decades, research scientists have only begun to address their basic mechanisms.

The International Conference on the Biological Mechanisms of Occupational Lung Disease (ICBMOLD) has the specific objectives of addressing these basic mechanisms. Speakers and contributors of international caliber have been selected and have agreed to participate in this 7th annual research conference that will address the specific areas of biological mechanisms of occupational lung disease.

Occupational Lifting and Low Back Muscular Fatigue

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Musculoskeletal Injuries
1 R01 OH01929-01A1
01/01/86 - 06/30/87
$68,026 ($68,026 Cum)

Objectives

This project will investigate muscular fatigue as a causative factor in low back injuries with the long-term objective of better evaluating, treating, and preventing low back disabilities. The aims of this project are: (1) during a continuous, repetitive, fatiguing lifting task, to describe the changes which occur in the instantaneous fatigue state of muscles of the low back and in selected kinematic and kinetic features of lifting; and (2) during this same lifting task, to determine the relationship between fatigability and the fiber composition of the muscles of the low back.

Methodology

Thirty normal subjects will undergo a screening medical examination and lift strength testing. Under direction of computer controlled visual and auditory cues, each subject will then work at a repetitive lifting task having load and frequency parameters which are known (from psychophysical studies) to be fatiguing. During repetitive lifting the following variables will be monitored: (1) flexion/extension motion of the lumbar spine; (2) vertical acceleration of the load; and (3) myoelectrical activity from five locations over the paraspinal muscles. The task will continue until predetermined fatigue criteria are reached (or the subject requests to discontinue the experiment). Following testing, fiber composition of the low back muscles will be determined by muscle biopsy. Data analysis will consist of determining the relationships among the variables as stated in the specific aims.

Progress and Accomplishments

Data have been collected and analysis is underway.

Significance

The significance of this project is two-fold. First, it will provide a description of the changes in selected kinematic and kinetic features which occur as the requirements of a lifting task exceed the capabilities of a worker. Secondly, it will examine whether the relative fiber composition of the low back musculature is a predisposing factor in the onset of fatigue and alterations in lifting technique.

Back Pain in Industry: A Prospective Epidemiologic Study

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Musculoskeletal Injuries
5 R01 OH02084-03
12/01/84 - 03/31/88
$176,745 ($488,597 Cum)

Objectives

A prospective epidemiologic study of back pain in the industrial population of the Allen-Bradley has entered a third year. This large scale study was designed to characterize indicants of individual and occupational "predisposition" to low back pain and to define rates of occurrence of low back pain. Specific variables addressed as potential risk factors included attributes of the individual, characteristics of the work environment and characteristics of occupational tasks and recreational activities. Correlations of these variables have been sought to "episodes" of low back pain (resolution without specific treatment), "cases" of low back pain for which treatment is sought and the frequency and duration of work loss. Remedies sought by the employee have been catalogued and major descriptive outcomes of the study have included prevalence rates, annual incidence rates and recurrence rates for the total population of employees and for various demographic subgroups in different job categories. Identification of major risk factors may enhance the likelihood of successful implementation of appropriate preventive strategies.

Methodology

Employees at the Allen-Bradley Company entering the study have completed questionnaires administered by nurse-coordinators at the work site. Questionnaires characterize individual demographic characteristics, off work activities, pertinent medical history relating to low back paid and the employees' perception of job tasks. Anthropometric measurements have been obtained at the time of entry into the study. Rates of occurrence of "episodes" of low back pain have been analyzed by annual follow-up questionnaires and "cases" have been identified at the time of presentation to the Medical Department of the Allen-Bradley Company. Characterization of occupational environment and work tasks has continued throughout the second and third years of the study. A number of cross sectional and logitudinal associations of risk factors to outcome variables are being accessed, as univariate and multivariate statistical techniques have been employed.

Progress and Accomplishments

Entry questionnaires and anthropometric measurements have been completed in 2,668 employees and 2,127 first annual questionnaires have been completed. Second annual questionnaires have been completed by 766 employees and ergonomic analysis of job site and work task has been carried out on 698 selected individuals. Short form questionnaires completed by employees presenting to the Medical Department with episodes and cases of back pain have been collected and departmental records have been abstracted. Prevalence of back pain and stiffness was noted to be 86%. Rates of occurrence and recurrence were found to vary substantially between back sites (upper/lower; midline/paramedian). The group with exclusive midline low back pain has been individually analyzed to exclude the possible confounding affect of pain at other sites which is to be analyzed as an independent risk factor. Risk factors identified in the Allen-Bradley population have included the male gender and parameters of body frame size including wrist and elbow bredth. A peak of prevalence in the fifth decade was identified in both males and females and was significantly associated with higher weights in males. The waist/hip ratio was more strongly associated with back pain than was either measure in isolation. Relative risk rates were dramatically higher for recurrence of back pain than for development of a first episode. Prevalence rates were not statistically significantly different between white and blue collar workers. The likelihood of development of initial episodes of pain was significantly higher in individuals who reported either sitting or standing for more than six hours per day, however, recurrence rates of back pain did not appear to vary significantly on the basis of a preliminary classification of job tasks. Estimates of incidence of prevalence of low back pain in non-participants were obtained by anonymous questionnaires.

Significance

Pain is among the most common of human afflictions and back injuries are among the most frequent of work related diseases. Costs are reflected both in terms of human suffering and in immense economic losses to industry and to society. Epidemiologic data on low back pain have not been extensively developing in the United States; prevention of back pain and prevention of the progression of back pain to disability may become more likely through identification of risk factors.

Wrist Orientation Effect on Grip Strength and Endurance

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Musculoskeletal Injuries
1 R03 OH02178-01A1
09/29/87 - 09/28/88
$23,866 ($23,866 Cum)

Summary

This study is intended to provide basic data about some factors that may affect the ability of an individual to apply a gripping force. The aim of this study is to determine the effect of wrist orientation on grip strength and grip endurance. Twenty men and 20 women will be used as subjects. They will be asked to apply a maximum static force to a grip dynamometer and maintain a maximum contraction until their gripping force falls to 70% of the peak. The duration of the grip force application will be used as a measure of fatique time or endurance. Peak forces and linear impulses (to determine how the gripping force changed) of the gripping forces at various wrist orientations will be measured and related to the maximum values generated by each subject. Wrist orientations will vary by 15-degree increments in wrist angle in four directions: plantar flexion, dorsiflexion, ulnar deviation and radial deviation. Relative changes in peak force, fatique time and linear impulse will be evaluated for all the subjects at all wrist positions and also compared between the sex groups. This type of basic data will be of use to those people who are concerned with musculoskeletal disorders. They will be able to suggest changes in the manner in which a tool is held in order to decrease the susceptibility of an individual to musculoskeletal disorders.

Maximum Acceptable Lifts in a Sitting Position

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Musculoskeletal Injuries
1 R03 OH02229-01
05/01/86 - 08/31/87
$20,693 ($20,693 Cum)

Objectives

Despite the recent automation and mechanization of work, it is still necessary in many industrial settings to perform some form of manual materials handling. Musculoskeletal disorders (such as low back pain, muscle soreness, tendonitis, bursitis, wrist and joint injuries, etc.) associated with repetitive exertions represent a major cause of lost work time and compensation costs. Over the past twenty to thirty years most of the research in this area has concentrated on injuries of the low back. This knowledge led to the publication of a NIOSH Technical Report on manual lifting in 1981. Curiously, the guide deals only with lifts in the sagittal plane from a standing position. In a modern industrial setting, many jobs such as production assembly or packing jobs require lifts from a seated position. People who sit for extended periods often experience more back pain than those not so constrained. It has been shown that the stresses on the lumbar verterbra in a sitting position exceed those generated from a standing position. Despite this knowledge, little information is available concerning lifting limits from a sitting position. Knowledge of these results would be invaluable in the design of the workplace and the evaluation of the strain imposed by work tasks. The purpose of this project is to determine the maximum acceptable load that can be safely lifted with two hands within the normal reach limits from a sitting position and to compare those values with the maximum acceptable weight in a standing position.

Methodology

The psychophysical methodology is being used to determine the maximum acceptable weight that college-age students will lift in each of four positions: (1) seated, two-handed, symmetrical lift from a table, forward 38 cm., (2) seated lift from 30.5 cm. below table height to table height (85 cm.) with a 90 degree twist, (3) standing, two-handed, symmetrical lift from the table, forward 38 cm., and (4) standing, vertical lift from 86 cm. to 134.5 cm. Subsequent to a training period, subjects lift a tray with slotted handles at the rate of 1 or 4 lifts/minute. Each subject adjusted the weight of the tray to their own preference by adding or removing flat pieces of lead over a 45 minute period. The weight of the tray, heart rate and perceived exertion are measured at 15, 30 and 45-minutes. Oxygen consumption is measured during the last 5 minutes of the 45 minute period. An indication of muscle strain will be determined from electromyographic analysis (EMG) of the back, shoulder, and abdominal muscles. The analysis of the data will determine if the amount of the weight chosen by the subjects is influenced by the lifting position. In addition, the physical strain will be assessed by the heart rate response, oxygen consumption and EMG. The perceived exertion indicated the overall assessment of the job by the subject.

Progress and Accomplishments

Because there was some delay in obtaining the EMG equipment, Exp 1 was completed without any EMG data. This study used 8 male, college-aged students as subjects. As was expected the amount of weight lifted 4 times/minute was significantly less than that lifted 1 time/minute (approximately 2 kg). No significant differences in the amount of weight lifted existed between the sitting-forward lift and the sitting-twisting lift. Likewise there was no significant difference between the standing-forward lift and the standing-vertical lift. However, the loads lifted during both standing positions were significantly greater than during either sitting position. The acceptable weight for the standing-vertical lift was approximately 22% and 14% greater than the sitting-twist and sitting-forward lifts, respectively. When averaged across positions and frequencies, the maximal acceptable weight for standing lifts was 16% greater than for sitting lifts. This study also provided data relative to the oxygen consumption, heart rate, and perceived exertion of the subjects during the lifting tasks.

With the arrival of the EMG equipment, a second group of male subjects (n=8), was tested at 2 and 6 lifts/minute. Data analysis is incomplete at the time of this report. Somewhat surprisingly, the data available suggested no significant difference in the tray weight lifted between 2 and 6 lifts/minute even though heart rate was significantly higher at 6 lifts/minute. In this portion of the study the maximum acceptable weight for the sit-twist task was greater than for the sit-forward position (approximately 2 kg). The weight that was acceptable to the subjects in both standing positions exceeded the acceptable weight while sitting by approximately 17%. This was similar to the findings of the first study. The standing verticl lift was perceived as less stressful to the lower back than the other lifting tasks. The EMG data are not yet available.

Significance

This project will provide data concerning the maximum acceptable weight that can be comfortably lifted from a sitting position using the psychophysical methodology. This data is not currently available. More importantly, by comparing the acceptable weight in both sitting and standing positions in the same group of subjects, we can arrive at a percentage change which can be applied to the volumes of standing data that is already available. This will greatly decrease the number of studies which need to be performed in this area in order to provide acceptable guidelines for design engineers.

Publications

Yates JW, Karwoski W: Maximum Acceptable Lifting Loads During Seated and Standing Work Positions. Applied Ergonomics 18.3, p 239-243, 1987

Mixture Model for Low Back Pain Rehabilitation

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Musculoskeletal Injuries
1 R03 OH02287-01
09/01/86 - 08/31/87
$23,464 ($23,464 Cum)

Summary

Because of the lack of knowledge of true causes of low back pain (LBP), a great variety of non-operative treatment methods have been recommended and tried. Recently a new regime, called "back school" was introduced for the purpose of rapidly and economically returning a patient to full functional fitness and normal occupational activities. Assuming that this functional and vocational restoration is a goal of a rehabilitation program, it is apparent that early recovery and higher success rates are major concerns. Hence it is important to consider these two parameters in evaluating any individual treatment modality or the rehabilitation program as a whole.

It is proposed to construct a statistical model (mixture model) that can be used for the investigation of the recovery process of LBP patients in a rehabilitation program. The model provides (i) the percentage of patients who will be recovered, and (ii) the rate of recovery of function. The model will then be used to identify the factors that influence the process of LBP rehabilitation significantly. Approximately 400 LBP patients who participated in the program offered by one of the large urban rehabilitation centers will be studied. In this study "recovery" is defined as a return to work at least on a half-time basis.

A Carcinogen/Mutagen Fetector for HPLC

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Occupational Cancers
2 R01 OH01331-04A1
08/01/82 - 08/30/87
$160,935 ($482,560 Cum)

Objectives

The primary objective of this project was to develop a detector for high performance liquid chromatography that is selectively sensitive to chemical carcinogens. Since this detector is based on the rate at which excess electrons attach to chemical carcinogens, a secondary objective was to establish more firmly the validity of the correlation between K_e, the rate constant of attachment of excess electrons to a chemical, and the carcinogenicity of that chemical. Since K_e's can be measured only in nonpolar liquids, another objective was to demonstrate that analyses of complex mixtures of K_e-carcinogenicity correlation and to establish if a short-term carcinogen-screening test based on this correlation could be used to complement short-term bioassays that are currently in use.

Methodology

The technique upon which the carcinogen detector is based is pulse conductivity, and using the detector under development in conjunction with HPLC can be viewed as repetitively sampling the K_e of a test solution at a rate of 50 cycles/second as the solution is pumped through the detector. Single-shot measurements of K_e are routinely conducted in our laboratory using a Van de Graaf accelerator to generate a 15 ns pulse of 1 MeV electrons that irradiate the test solution and produce secondary excess electrons. The attachment of these electrons to the test chemical is monitored via the decay of the conductivity as high-mobility excess electrons attach to a test chemical and are thereby converted to low-mobility anions. The difference in conductivities between each sample component and the pure solvent as both flow through two separate ion chambers that comprise the detector is recorded as a function of time to yield an HPLC chromatogram of the relative quantity of each electrophilic component in a sample of a mixture of chemical carcinogens.

The second methodology used in this project is HPLC which is the most versatile means of separating mixtures of chemicals prior to their elution through a detector that measures some physical or chemical property of each eluite. Since this measurement with the carcinogen detector necessitates the use of a nonpolar eluent, the HPLC technique used is restricted to normal-phase chromatography. However, reversed-phase HPLC, liquid-liquid and solid-phase extractions as well as Soxhlet extraction are used to fractionate complex mixtures into components that are amenable for sampling with the detector.

Progress and Accomplishments

Effort during the one-year renewal of funding of this project was directed toward those areas in which the Safety and Occupational Health Study Section (SOHSS) expressed the greatest concern or interest in their review of the request for continuation of this project in October 1985. "One of the more interesting aspects of the work" that they cited was "determining the theoretical basis of the K_e-carcinogenicity correlation" and "particularly interesting" was the "question of why K_e detects procarcinogens". Significant progress in this area was made which is evident in References 1-4 of the listing of publications.

The same references also provide additional validation of the K_e-carcinogenicity correlation and demonstrate the K_e can be used to identify chemical carcinogens with a greater sensitivity and specificity than the most widely used carcinogen-screening bioassay, which is the Ames Salmonella/microsome test. For example, of the 152 carcinogens and putative noncarcinogens that have been screened with the K_e and Ames tests, the K_e test correctly identified 79% of the chemicals whereas the Ames test accuracy was 63% (2).

Effort was also directed toward demonstrating that the concern of the SOHSS related to the use of normal-phase HPLC to analyze complex mixtures was groundless. References 5-8 illustrate our progress in this area in which separations of polyaromatic hydrocarbons in coal tar oil and diesel emissions by normal-phase HPLC are described.

Significance

The carcinogen detector and the K_e test upon which it is based could be an invaluable tool to industrial hygienists in identifying occupational biohazards and in assessing the risk of exposure of workers to such biohazards. In addition to this obvious application of the detector and the K_e test, the most significant aspect of this work may prove to be the implications of the K_e carcinogenicity correlation to providing a better understanding of the initiation step in the multi-stage process of carcinogenesis.

Publications

Isildar M, Bakale G: Radiation-Induced Mutagenicity and Lethality in Salmonella Typhimurium. Proceeding of the Seventh International Congress of Radiation Research. JJ Broerse, GW Barendson, HB Kal, AJ van der Kogel, Eds., p. B4-19 M Nijhoff, Amsterdam, 1983

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Isildar M, Bakale G: Comparative Lethal Effects of UV and Ionizing Radiation in Ames Tester Strains of Salmonella. Radiat Res 103:461-465, 1985

Bakale G, McCreary RD: A Physico-Chemical Screening Test for Chemical Carcinogens. Carcinogenesis 8, 253-264, 1987

Production and Fate of Hydroxamic Acids in Hepatocytes

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Occupational Cancers
5 R01 OH02027-03
08/01/84 - 07/31/87
$73,722 ($201,902 Cum)

Objectives

The objective of this program is to determine the extent to which thiamine-dependent enzymes participate in the bioactivation of C-nitroso aromatic chemicals in primary cultures of rat liver hepatocytes. Specific aims relevant to the elucidation of this objective are to 1) identify the hydroxamic acids produced from selected nitroso substrates by hepatocyte cultures, 2) determine the metabolic fate of such hydroxamic acids in hepatocytes and 3) determine the relative genotoxicity of the various types of hydroxamic acids of interest, particularly with respect to covalent binding to nucleic acids and protein.

Methodology

Rat hepatocytes in primary culture are prepared under aseptic conditions and used within four days. Chemical substrates containing the C-nitroso group on an aromatic ring are added to such cultures, and their metabolic fates are followed. Particular effort is made to determine the metabolic production of certain hydroxamic acids, which are indicative of the action of particular thiamine-dependent enzymes on nitroso substrates. Authentic chemical reference standards are employed to confirm the production of the metabolites of greatest interest. The biochemical fates of the hydroxamic acid metabolites are also examined by such techniques. Particular effort is made to determine the extent of metabolic activation of the hydroxamic acids, and subsequent covalent binding of them to cellular molecules such as DNA and RNA.

Progress and Accomplishments

Rat hepatocytes were employed in studies to determine the fate of selected nitroso aromatic chemicals, particularly with respect to their conversion to hydroxamic acids. Of greatest significance was our observation that a small percent of each nitroso aromatic was converted to the glycolyl-type of hydroxamic acid. This confirmed the original hypothesis of the grant; however, funding was discontinued by NIH before these results could be repeated for statistical purposes, and it is unlikely that the results will be published because they have not been sufficiently confirmed.

^{14}C-Labelled hydroxamic acids in the 2-aminofluorene series were synthesized and investigated for their ability to undergo binding to hepatocyte nucleic acids. In cell suspension studies, it was observed that both the acetyl and glycolyl-type hydroxamic acids were bound equally to DNA and RNA. Although this finding was suggestive that similar bioactivation pathways were operating on both types, subsequent in-vitro studies indicated that this was not the case. The N-acetyl hydroxamic acid was bound to nucleic acid via the actions of N,O-acyltransferase and sulfation reactions in agreement with prior reports from other research groups. On the other hand, we observed that nucleic acid binding of the N-glycolyl hydroxamic acid was catalyzed primarily through O-sulfation and via a microsomal enzyme. N,O-Acyltransferase was strongly inhibited by the glycolyl hydroxamic acid, and the inhibition was of the suicide-type. It was concluded that the nature of the N-acyl group has a strong effect on the mechanism by which hydroxamic acids undergo bioactivation.

The glycolyl hydroxamic acids in the 2-aminofluorene and 4-aminobiphenyl series were found to be approximately as potent as the analogous N-acetyl hydroxamic acids in the Ames Assay. This is in general agreement with our results on nucleic acid binding in hepatocytes.

Significance

This study has confirmed the original hypothesis that certain thiamine-dependent enzymes are involved in the production of structurally unique metabolites of nitroso compounds. Since the nitroso compounds are known intermediary metabolites of arylamines and related chemicals, it is expected that the unique hydroxamic acids derived from the action of transketolase on nitroso compounds will also be metabolites of the arylamines. This study has also provided strong evidence to indicate that glycolyl hydroxamic acids are just as genotoxic as are the more common acetyl hydroxamic acids. More importantly, the enzymatic mechanisms by which the two basic types of hydroxamic acids undergo bioactivation are considerably different. Such differences could have a strong effect upon the organ site of carcinogenesis by such chemicals. Even though the amounts of such hydroxamic acids that are produced are very small, it is possible that these minor metabolites play an important role in the overall toxicological properties of many industrial and agricultural chemicals.

Publications

Corbett MD, Corbett BR: Enzymatic N-oxidation of 4-nitroaniline. Biochem Arch, 1:115-120, 1985

Corbett MD, Corbett BR: The Reactions of C-nitroso Aromatics With α-oxoacids in Biological Oxidation of Nitrogen in Organic Molecules (JW Gorrod and LA Damani, eds.), Ellis Horwood Ltd., Chichester, p 400-408, 1985

Corbett MD, Wei C, Corbett BR: Nitroreductase-dependent Mutagenicity of *p*-nitrophenylhydroxylamine and its N-acety and N-formyl Hydroxamic Acids, Carcinogenesis, 6: p 727-732, 1985

Corbett MD, Corbett BR: Effect of Ring Substituents on the Transketolase-catalyzed Conversion of Nitroso Aromatics to Hydroxamic Acide, Biochem Pharmacol, 35: p 3613-3621, 1986

Lim LO, Corbett BR, Corbett MD: Irreversible Inhibition of the Cytosolic Metabolism of N-hydroxy-2-acetylaminofluorene by its Glycolyl Analog. Cancer Lett 37: p 205-211, 1987

Corbett MD, Lim LO, Corbett BR, Johnston JJ, Weibkin P: Covalent Binding of N-hydroxy-2-acetylaminofluorene and N-hydroxy-2-glycolylaminofluorene to Rat Hepatocyte DNA: *In Vitro* and Cell-Suspension Studies, Chem Res Toxicol, Vol 1, No 1, p 42-46, 1988

Occupational Cancer Surveillance: New Approaches

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Occupational Cancers
5 R01 OH02067-03
09/28/84 - 03/31/87
$258,679 ($636,463 Cum)

Objectives

The specific scientific aims for this project are:

1. To determine cancer risks by occupation and industry for black and white males and females in conjunction with detailed tobacco smoking history, socioeconomic status, and age at diagnosis by cancer type,
2. To determine cancer risk within specific occupations in major local industries, such as automobile manufacturing, construction, machinery manufacturing, and primary ferrous metals manufacturing,
3. To investigate work-related cancer risk by race, gender, socioeconomic status, age at diagnosis, and cancer site among persons who have never smoked cigarettes, pipes, or cigars,
4. To perform methodologic analyses to:
   a. determine the effects upon risk estimates of including data obtained from surrogates for ill or deceased subjects,
   b. assess the accuracy of death certificate information regarding usual occupation and industry in comparison to the same information obtained by interview for study participants who have died, and
   c. develop study products that can be utilized by other investigators for occupational cancer surveillance and for individual studies in occupational epidemiology.

Methodology

This is a population-based study, conducted in the Detroit metropolitan area, of newly diagnosed black and white male and female cancer cases in the age group 40-48 at time of diagnosis for 9 forms of cancer: bladder, colon, eye, esophagus, liver, lung and bronchus, mesothelioma, rectum, and salivary glands. Cases are identified through the rapid reporting system of the population-based Metropolitan Detroit Cancer Surveillance System, a participant in the NCI SEER program. Cases matching the study protocol are interviewed by telephone within 2 to 6 months after diagnosis. This brief interview takes an average of 11 minutes to complete. It includes a health history, a smoking history, a complete lifetime occupational history (job title, industry title, description of job duties, length and dates of employment for each job), residential history, and demographic characteristics. For cases that are deceased by time of contact for interview or too ill for interview, interviews are being conducted with a surrogate. Case referent analyses are being performed, utilizing colon and rectum cancers as the referent group.

Progress and Accomplishments

Through December 1987 there were 15,294 cancer cases identified and enrolled in this study. Of these, 13,933 have been closed out and 1361 are in process for physician consent or respondent interview. Of the 13,933 closed cases, 13,199 interviews have been completed for a response rate of 94.7%. Physician refusals are 0.7% and respondent refusals are 1.9%. This study is the first to develop an occupational surveillance interview to complement routinely collected population-based cancer registry data in order to monitor cancer occurrence by occupation and industry and to develop new leads about occupational cancer risks. It was cited in a 1987 report by the National Academy of Sciences Panel on Occupational Safety and Health Statistics as an innovative approach to performing occupational surveillance on the basis of cancer registry data.

During the past year, an analysis of the correlation between usual occupation and industry and most recent occupation and industry revealed substantial mismatches between these two summary measures of an occupational history. The rate of mismatch varied by race and gender and by occupation and industry. Another analysis compared usual occupation and industry from the interview data with death certificate occupation and industry data for study subjects who are deceased. Match rates were compared for exact 3-digit 1980 U.S. Census Bureau codes and for groups of occupations and industries. Misclassification of occupation and industry on the death certificate in this analysis range from 30% to 50%, with variations by race and gender, marital status, number of years worked, occupation and industry categories, and age.

Preliminary analyses of cancer risks for usual occupations and industries for lung and bronchus cancer cases and for cases of urinary bladder cancer show some previously suggested risks, such as truck drivers, mechanics, and spray painters for lung cancer and plumbers and mechanics for bladder cancer. New high risk occupations and industries also have been identified, such as farmers and farming for both lung and bladder cancer, concrete and terrazzo finishers, assemblers, and coal mining machine operators for lung cancer and woodworkers and enlisted military personnel for bladder cancer.

Significance

Results obtained to date indicate that this study will make substantial contributions to both the methodology of research in occupational cancer epidemiology and the development of new hypotheses regarding occupational cancer risks in the workplace. The long-term goal of this study is to target specific groups of workers among whom the risk of cancer can be reduced by limiting or eradicating exposures to carcinogens encountered at work.

Publications

Swanson GM, Schwartz AG, Brown KL: Population-Based Occupational Cancer Incidence Surveillance--Utilization of the Telephone Interview. J of Occup Med 27:439-444, 1985

Illis WR, Swanson GM, Satariano ER, Schwartz AG: Summary Measures of Occupational History. A Comparison of Latest Occupation and Industry With Usual Occupation and Industry. Am J Public Health, in press

A Petroleum Solvent Mortality Study of Dry Cleaners

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Occupational Cancers
5 R01 OH02104-02
01/01/86 - 12/31/87
$123,518 ($246,723 Cum)

Objectives

This is a two-year solvent and exposure-specific mortality study of dry cleaners in Oklahoma. The objective of this study is to examine the association between workers' exposures to the primary solvents used in commercial dry cleaning plants and the distribution of causes of death among those exposed.

To achieve the research objective the specific aims will be:

1. To categorize each commercial dry cleaning plant by solvent use over time,
2. To identify a cohort of Oklahoma dry cleaners with known solvent exposures,
3. To categorize duration of exposure based on employment history,
4. To determine, among the cohort, the Standardized Proportionate Mortality Ratio (SPMR) and Standardized Proportionate Cancer Mortality Ratio (SPCMR) for selected causes of death to include all cancer, kidney cancer, and liver cancer,
5. To estimate latency in renal cell carcinoma, and
6. To identify high risk subgroups for future case findings and the measurement of the acute and chronic effects on the human kidney of exposure to hydrocarbons.

Methodology

The dry cleaning license applications from 1941-1985 available at the Oklahoma Dry Cleaners Board were reviewed and organized alphabetically by city. All data was coded and stored on our computer where plant, name, and the combination Cohort files were created. Each individual in the cohort of 3,978 owner/operators was categorized by solvent and durations of exposure from employment history.

Follow-up began to determine living status, obtain an address, phone number, date of birth and confirm information obtained from their license, if living. If deceased, the date and place of death is obtained through various sources, and their death certificate is requested and coded according to the Instruction Manual for Classifying the Underlying Cause of Death. A questionnaire is sent to obtain information on date of birth, Social Security number, smoking history and to verify exposure data if an address is recorded.

To calculate ratios, we will use age-sex-race and cause specific mortality for U.S. males from 1941-1985, and for Oklahoma white males from 1950-1985 as standards for comparison. We will calculate SMR's by solvent category, specific solvent and duration of exposure using NIOSH Life Table Analysis, Version E. The significant differences between observed and expected numbers of deaths will be tested using a Poisson Exact Statistics.

Progress and Accomplishments

Follow-up of the cohort was very productive early in the study time utilizing current phone books and accessible death tapes, but has slowed as these resources have been exhausted. To date, a total of 2,943 cohort members have been identified with confirmed status. Mostly from telephone contacts, 1,649 people (42%) are alive. A total of 1,294 (33%) are reported dead, and of these, 733 death certificates have been obtained and 334 are now being requested. Other deaths have incomplete information on date and place of death. After confirming an address, 1,451 questionnaires were sent out, of which 422 have been returned. The number of cohort members yet to be categorized total 1,035 (25%).

Preliminary results utilizing Proportionate Mortality Ratios (PMRs) show no excess in overall cancer mortality (PMR = 1.04, CL = 0.87-1.25). However, a number of specific cancer sites were found to be elevated. A significant excess was found for Cancer of the Pancreas (PMR = 1.87), CL = 0.9964-3.2035), Malignancies of the Respiratory System (PMR = 1.34, CL = 0.9977-1.7620), Malignancies of the Trachea, Bronchus and Lung (PMR = 1.31, CL = 0.9644-1.7465), and elevation was noted for Malignancies of the Urinary Organs (PMR = 1.48, CL = 0.7381-2.6495), including Kidney Cancer (PMR = 1.75, CL = 0.5691-4.1091) and Bladder Cancer 9PMR = 1.3, CL = 0.4777-2.8477). Also, Malignancies of the Skin were elevated 9PMR = 2.66, CL = 0.8633-6.2331). Other results show increases in Diseases of the Genito-Urinary System 9PMR = 1.46, CL = 0.76-2.56), including Acute Nephritis (Obs. = 3. PMR = 7.55, CL = 1.56-22.08) and Transportation Accidents (Obs. = 16, PMR = 1.42, CL = 0.81-2.32).

Significance

In human studies, definitions of exposures, exposure levels, and durations of exposure have been difficult to specify. The magnitude of the numbers of workers exposed to petroleum fractions, however, necessitates that any increased risks found in selected epidemiologic and animal toxicological studies be recognized, and their actual impact on worker health and safety be specifically assessed. Stoddard solvent is the principal petroleum hydrocarbon used in the dry cleaning industry since 1928 and is a petroleum fraction that encompasses the hydrocarbons most suspect of adversely affecting human health.

This study will show the mortality among a petroleum solvent exposed population and the dose-response relationship. With the forty-four year follow-up period, we will be able to have a description of dry cleaning solvent use trends over time, and also give an inference to other hydrocarbon exposed occupational cohorts. Additional important information will be provided on the risk associated with specific exposure to perchloroethylene, although the numbers in our cohort exposed to this solvent are small. A contribution to follow-up procedures will be provided with these methods of follow-up when Social Security number and date of birth are lacking.

Publications

Petrone, RL: Cancer Mortality Among Petroleum Exposed Oklahoma Dry Cleaners, a doctorate dissertation, University of Oklahoma, 1987

Petrone, RL, Asal, NR, Coleman, R: Cancer Mortality Among Petroleum Solvent-exposed Oklahoma Dry Cleaners, Am J of Epidemiology 126 (4): 743 October 1987

Urinary Excretion of Modified Nucleosides

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Occupational Cancers
5 R01 OH02122-03
08/01/85 - 07/31/88
$50,323 ($246,432 Cum)

Summary

Transfer RNA (tRNA) is a complex biomacromolecule containing a large number of modified bases. The modifications include methylation by several specific enzymes. These are hyperactive in malignant tumor tissue, which therefore contains tRNA that are different in structure from those present in normal tissue; they also have a high turnover rate. It is known that patients with cancer excrete in their urine elevated levels of such modified nucleosides (tumore markers). We have found abnormal levels of modified nucleosides in patients with asbestos related malignant mesothelioma, and we have shown in a feasibility study of individuals selected from the proposed study population but without clinical evidence of cancer that asbestos insulation workers, with a history of long-term exposure to asbestos and thus at high neoplastic risk, altered nucleoside excretion with appreciable prevalence. In the proposed investigation we will measure levels of modified nucleosides in the urine of 1,000 asbestos insulation workers with a history of 30 years or more from first onset of exposure. We will investigate whether the urinary excretion profile of nucleosides may be predictive of subsequent malignant disease. One objective of the proposed study is therefore to investigate the usefulness of modified nucleoside levels in identifying individuals at high neoplastic risk. Extensive clinical and laboratory information is available on the proposed study population. The nucleoside pattern will be characterized for the entire population, and a matrix of intercorrelations between nucleosides and pertinent medical and laboratory information will be generated. Intra group differences will be evaluated for each nucleoside with respect to classification variables reflecting health affects of asbestos associated disease. The principal analysis of the proposed investigation will include prospective surveillance of all examined in terms of their mortality experience between 1984 and 1986. For this purpose we have developed a mechanism by which we will be made aware of the death of any individual in the study population. We will then study the association between the initial nucleoside excretion pattern and the mortality experience. Such information will provide important information about the significance of nucleoside levels and their capacity to serve as predictive markers for future malignant disease. Clarification of this may have important implications for prediction of occupational cancer.

Feasibility Study for an Occupational Cancer Data Base

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Occupational Cancers
1 R01 OH02284-01
09/28/87 - 09/27/89
$19,540 ($19,540 Cum)

Summary

This is a pilot study of a method for collecting brief, standardized data on the usual occupation, industry and smoking habits of cancer patients included in the California Tumor Registry (CTR). The CTR is mandated to cover the entire state by 1990. The long-term goal is the inclusion of usual occupation, industry and smoking as variables (along with the currently standard variables of age, race and sex) in the routine monitoring of cancer incidence in California, for the purposes of cancer surveillance and the generation of etiologic hypotheses. Four hospitals in Contra Costa County have been chosen as test sites for the pilot study.

Approximately 250 patients, aged 25 and over, newly diagnosed or given their initial treatment at the test hospitals during a test period of 3-4 months, will be given a brief questionnaire, self-administered if possible, at the time of admission. A chronological work history and brief smoking history will be obtained by telephone from a random sample of 100 of those patients, to validate the brief questionnaire information. Occupational, industry and smoking information recorded in the medical charts of the sample of 100 patients will be reviewed and compared to the information obtained from the self-administered questionnaire and the telephone history. The type of job represented by the usual occupation and industry will be compared to the entire spectrum of jobs held by the patient. From this will be determined the proportion of patients who would be misclassified as to their potential for hazardous job exposures if they were classified only on the basis of their usual job. The proportion of patients from whom completed questionnaires could be obtained, will be compared to all cancer cases diagnosed in the test hospitals during the test period. The cost effectiveness of the three modes of data collection will be compared. The results of this project will be used to design the most cost-effective method of collecting occupation, industry and smoking information on all cancer patients in California.

Factors Modifying Diesel Particulate Composition

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Occupational Cancers
5 R01 OH02351-02
09/27/85 - 11/30/88
$39,331 ($108,362 Cum)

Objectives

Ceramic particle traps are being developed for use as diesel exhaust emission controls for over-the-road as well as heavy-duty diesel engines used in underground mines. These devices collect entrained diesel aerosols, and on reaching the regeneration temperature, catalyze combustion of trapped materials. Although they are reported to reduce the mass of emitted soot, little is known about the physical and chemical properties and biological activity of the particulates they transmit. While these control devices reduce the opacity of tailpipe emissions, and thus are desirable aesthetically, the possible impact of this technology on human health needs evaluation. This is particularly true for occupational settings such as underground mines where workers may be exposed to diesel fumes at twenty-five times the levels found in congested urban canyons with proportionately greater risk.

The long-term project objective is to assess the relative hazard to human health of particulate emissions from diesel engines with and without ceramic particle traps. The study will focus on cancer risk as opposed to non-malignant respiratory effects.

The comparative risk assessment will be accomplished through the following specific aims: 1) collection of particulate samples from commercial heavy-duty mining engines under simulated mining conditions; 2) determination of the mutagenic potency of the diesel particle soluble organic fraction (SOF) with the Ames Salmonella test; 3) quantitation of known carcinogens and mutagens in particulate SOF; 4) definition of the operating modes which contribute most to the production of chemical carcinogens under steady-state sampling conditions; and 5) further examination of the chemical composition and biological activity of particulate emissions under conditions of transient diesel engine operation.

Methodology

Diesel particle samples (provided by the U.S. Bureau of Mines) were collected from a diesel test cell consisting of a commercial diesel mining engine, an engine dynamometer, an exhaust dilution tunnel and a particle collection system. A water-cooled, 6-cylinder Deutz F6L 912 W mining engine was operated under transient conditions in a research duty cycle representing typical load-haul-dump (LHD) mining equipment operation. The particles collected in this system accurately simulate tailpipe emissions. The variables studied in this budget period were the ceramic particle trap, a barium smoke suppressant fuel additive (Lubrizol 565), altitude, and a water scrubber, each of which may affect diesel exhaust particulate emissions.

Progress and Accomplishments

Carcinogen Analysis. An analytical procedure for the routine quantification of polycyclic aromatic hydrocarbons (PAH) in diesel exhaust particulate matter was developed and validated. This procedure complements a method to analyze nitro-substituted PAH developed in the first year of NIOSH project support.

Ceramic Particle Trap. The ceramic trap was effective at removing particulate matter (87% for a steady-state LHD mining cycle); particulate SOF (90% for the duty cycle); and exhaust mutagenicity (>93% in modes examined) when used on an air-cooled engine. The air-cooled engine was "cleaner" in general than an equivalent water-cooled type studied in the previous year, possibly due to higher combustion temperatures and efficiency. On the air-cooled engine exhaust, particles were high in SOF and those transmitted by the trap did not show elevated mutagenic potency as found with the water-cooled engine.

Barium Smoke Suppressant. A commercial barium fuel additive is currently used in underground mines because of its ability to reduce the opacity of diesel exhaust. Our studies indicate that the barium additive: 1) does not significantly reduce the mass of soot or particulate SOF emitted; 2) increases exhaust mutagenicity; and 3) does not affect the concentration of carcinogenic PAH compounds in engine exhaust.

Significance

The development and thorough evaluation of emission control devices for diesel-powered mining equipment is critical in protecting the health of underground miners. Recent inhalation studies in experimental animals demonstrate that chronic exposure to diesel particles at elevated levels (e.g., 3.5 - 7 mg/m^3) causes lung cancer (Mauderly et. al., Fund. Appl. Tox. 1987, 9, 208). Diesel exhaust particulate exposures in miners also are expected to be high; diesel particle concentrations as high as 1.2 mg/m^3 have been measured in underground mines although few in-mine measurements have been reported.

These studies demonstrate the efficacy of the ceramic trap on air-cooled diesel engines on which very little data is available. These data demonstrate that the ceramic particle trap may be useful for improving the quality of mine air, and substantially minimizing both the exposure of miners to diesel particulate and its potential adverse health effects. The present studies also suggest that the barium smoke suppressant, while lowering exhaust opacity does not reduce chronic health hazards of exposure to diesel exhaust.

Publications

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Detection of Adriamycin on Surfaces and Skin

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Occupational Cancers
1 R01 OH02413-01
09/30/87 - 09/29/88
$59,460 ($59,460 Cum)

Summary

Cancer patients receive chemotherapy in a variety of treatment settings: hospital inpatient, hospital outpatient, and physician office. The drugs may be received by the administering personnel premixed from a pharmacy or supplying company or mixed on-site just prior to administration. Contaminated waste, bed linens, vomitus and excreta may be handled by a number of persons involved in either treatment, patient care or facility maintenance.

Interest in potential adverse health effects among health care workers has focused on pharmacists who mix drugs and nurses who mix and/or administer drugs. All reports include surrogate measures of exposure, i.e., total doses handles, or no exposure data. A systematic survey of pharmacists, nurses who administer and other health care workers with potential for drug contact has not been undertaken.

The proposed study will provide data to compare the degree of dermal contact with adriamycin experienced by health care personnel in hospital and non-hospital settings. The potential population at risk includes pharmacists, i.v. therapy administration personnel, physician office administration personnel, patient care nurses, maintenance workers and hospital laundry workers. Surfaces in the work area and employees skin will be surveyed with an instrument developed at the University of Cincinnati. The device allows the detection of concentrations of adriamycin as low as 0.001 mg/ml, three orders of magnitude lower than the administration concentration.

Industrial hygiene measures to reduce exposure will be developed for all groups found to be at risk of dermal contact with adriamycin.

Development of a Human DNA Adduct Monitoring Method

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Occupational Cancers
7 R03 OH02238-02
05/01/86 - 05/31/87
$21,206 ($21,206 Cum)

Objectives

DNA adducts result from covalent binding by agents to the bases of DNA. Monitoring adduct formation in carcinogen exposed persons may be a good dosimeter of the risk of genetic disease because individual differences in absorption, distribution, excretion and DNA repair are effectively integrated. DNA binding is likely to be a relevant end point for genotoxicity.

The post-labeling method, developed by Kurt Randerath, has potential to be used as a tool to monitor DNA adducts in humans. No radioactive carcinogen is required and the method combines thin layer chromatography with scintillation counting for simplicity and sensitivity. The method can be used to analyze different, but related compounds, so it is adaptable to various exposure situations.

To be accepted as a human monitoring tool, the post-labeling method should first be validated with other tests of genotoxicity in animal models. The quantitation of post-labelling and standard scintillation counting will be compared in mouse liver using labelled benzidine. The kinetics of benzidine adduct formation and removal (repair) will be studied. Then, the relationship between DNA adduct levels and subsequent chromosome aberrations will be determined in the liver of partially hepatectomized mice. The liver was chosen for those studies because it is the target organ of benzidine carcinogenicity in mice.

Methodology

DNA isolated from mouse liver is degraded to the 3'-phosphonucleotides, which are then labelled with ^{32}P from (Y-^{32}P) ATP. Normal nucleotides are resolved from the adducts by thin layer, ion exchange chromatography. Because of post-labelling's requirement of only microgram quantities of DNA, adduct and chromosome analyses could be performed from the tissue of each animal.

Progress and Accomplishments

The correlation between DNA adduct levels and chromosome aberrations has been established. On the basis of individual animal livers the correlation between the two parameters is 0.624. When dose group analysis is performed, the correlation is 0.94. The shapes of the dose response curves for adducts and aberrations implies that a third process underlies both. The study of the kinetics of adduct formation indicates that metabolic activation of the procarcinogen is the most likely explanation. The time at which adduct levels peak is dose-dependent. Adduct levels as low as 1 adduct in 37 million normal nucleotides could be detected reproducibly.

Significance

These data indicate post-labelling has real potential to be used to monitor DNA adduct formation in exposed humans. Adduct levels are casually associated with chromosome aberrations and predict this significant genetic effect. Adducts could be detected at levels approaching what might be expected to result from human exposure. These studies also indicate that metabolic differences between individuals may affect adduct formation.

Publications

Talaska G, Au WW, Ward Jr. JB, Randerath K, Legator MS: The Correlation Between DNA Adducts and Chromosome Aberrations in the Target Organ of Benzidine Exposed, Partially-hepatectomized Mice. Carcinogenesis, 8 (12), in press

Cytogenetic Assessment of Occupational Exposure To Antineoplastic Agents

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Occupational Cancers
5 R03 OH02243-02
05/01/86 - 04/30/88
$21,590 ($42,682 Cum)

Summary

This study proposes to evaluate the utility of measuring the effects of low level, long term occupational exposure of hospital personnel to antineoplastic agents through conventional cytogenetics (chromosomal breakage) and to attempt to correlate those measurements with micronuclei frequency.

The study population includes nursing personnel evaluated for actual occupational exposure to antineoplastic agents through admixing and administering chemotherapy drugs. Based on documentation of exposure, nursing personnel will be placed in one of three exposure categories: no known exposure, low exposure or high exposure.

Patients diagnosed, but not yet treated for breast cancer will serve as the second study population group. Patients will be both no exposure - negative controls (pre-treatment) and very high exposure - positive controls (post treatment).

All study subjects will be interviewed to identify possible confounding factors of exposure, age, smoking and occupational or environmental exposure to genotoxic agents. Blood samples will be collected from all subjects (single sample from nursing personnel, pre-treatment and post treatment sample from patients).

Peripheral blood lymphocytes will be analyzed for determination of frequencies of chromosomal breakage and micronuclei.

Frequencies will be compared between exposure groups to attempt to identify the absence or presence of a dose response relationship between exposure to antineoplastic agents and chromosomal damage. Corelation will also be assessed between the frequency of chromosomal breakage and the frequency of micronuclei. Significant positive correlation coefficients would indicate the appropriateness of estimating chromosomal damage through the more efficient and cost effective technique of micronucleus methods.

Explosion Hazards Related to Grain and Feed Dusts

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Traumatic Injuries
5 R01 OH01122-06
01/01/81 - 12/31/87
$99,184 ($580,069 Cum)

Objectives

The purpose of this research project is to quantitatively characterize the explosion hazard represented by suspended and layered combustible dusts. While much testing concerning the explosive behavior of combustible dusts has been done for many years these results are apparatus dependent and provide no data which can be used in the computer modelling of scenarios applicable to large scale dust explosions. The results fail to characterize a fundamental physical quantity, the burning velocity, and none of them pertain to the secondary explosions which result from layered dust accumulations. Under this project the burning velocity for a suspended dust cloud and a resuspended layer of dust has been measured. Appropriate analytical efforts have also been made in order to explain these results.

Methodology

Two unique pieces of experimental equipment were developed under the grant in order to be able to measure the fundamental data required and to avoid the criticisms associated with previously acquired dust explosion test data which was obtained using standard test apparatus. The suspended dust combustion studies are conducted in the Premixed Turbulent Combustion Bomb (PTCB) which is a spherical, one cubic meter jet stirred reactor. The combustion process is initiated at the center of the well characterized, uniform turbulent dust cloud, and during its propagation toward the vessel wall appropriate measurements are made to characterize the burning process. The layered dust combustion studies are done in the Flame Acceleration Tube (FAT) which consists of three continuous segments of 3000 psi working pressure steel tubing each having a length of forty feet and an inside diameter of one foot. It is closed at one end and open at the other. A controlled thickness and width dust layer is placed along the bottom of the tube, and it is then ignited by the combustion of a presuspended dust cloud (primary explosion) in the first twelve feet of the closed end. The history of the resulting combustion process as it accelerates toward the open end is monitored using regularly spaced appropriate instrumentation. In both of these facilities the burning velocity and the post combustion conditions are measured as a function of parameters characterizing the dust and precombustion hydrodynamic conditions. For the analytical work being conducted with regard to the layered dust combustion the computational techniques originally developed by D.N. Chi and H.E. Perlee at the U.S. Bureau of Mines are being employed. The smoldering data reported on under the grant was collected using a specially developed Smoldering Wind Tunnel (SWT) which allows careful control of the ventilation of the fuel bed.

Progress and Accomplishments

Using the PTCB the laminar and turbulent burning velocities have been measured for two combustible dusts considering the influence of such variables as composition, concentration, size, moisture content, and turbulence intensity. The turbulence scale length has also been measured, and it has been determined that conditions within the PTCB are approaching those required for flame extinction. Additionally, the combustion characteristics of hybrid mixtures (gaseous and solid fuel together) have been examined, and it has been noted that for volatile solid fuels the addition of small amounts of dust can significantly extend the lower explosibility limit of the gaseous fuel. For less volatile dusts there is no extension of the lower explosive limit of the gaseous fuel, with the dust apparently acting as an energy sink. Using the FAT the burning velocity has been measured for combustible dusts considering in addition to the variables mentioned above for the PTCB layer parameters such as thickness, width, and continuity. The effect of oil treatment of the dust on its combustion characteristics has also been considered in view of its reported favorable effects with regard to facility housekeeping. If modelling of secondary dust explosions is to be verified analytical results duplicating data collected in different test facilities will be required. As such data has been obtained concerning the combustion of Pittsburgh Seam No. 8 coal dust in larger scale layered dust experiments at other facilities. Such measurements have also been made in the FAT. The smoldering results which were reported give the reaction velocity for two materials of industrial interest (grain dust and saw dust) the results of which were then closely approximated by a one dimensional analytical model.

Significance

For the first time turbulent burning velocity data is available for several dust air mixtures. For almost a century comparable data has been available for gaseous reactants. With this data it is possible to calculate previously measured dust explosion parameters such as K_{st} and P_{max}. The burning velocity can be used as input for yet to be developed computer programs which, as has been in the case of gaseous reactants, should allow the computation of flame development in complex geometric environments. With regard to layered dust combustion or secondary explosions actual data is now available as to what represents hazardous accumulations of dust. No longer do extrapolations have to be made using testing data obtained for suspended dust clouds on primary explosions. The agreement between the analytical and experimental results are encouraging in that it is not possible to test all industrial situations. In the past calendar year, 1987, dust explosions have continued to persist in the grain industry with 13 incidents producing 16 injuries. However, there were no deaths. On 31 December 87 in the Federal Register the Final Rule; Grain Handling Facilities was published. Some of the data collected under this grant is the only information pertaining to technical questions which this rule may raise.

Publications

Kauffman CW: Agricultural Dust Explosions in Grain Handling Facilities. SM Study #16, University of Waterloo Press, 1982

Kauffman CW, Nicholls JA: Dust Explosion Research at The University of Michigan. SM Study #16, University of Waterloo Press, 1982

Work-Related Burn Injury Incidence in New England

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Traumatic Injuries
1 R01 OH02241-01A1
05/01/87 - 04/30/88
$61,398 ($61,398 Cum)

Summary

A recent NIOSH Symposium on the Prevention of Work-related Diseases and Injuries (Atlanta, Georgia, May 1-3, 1985) stressed the need for information relating to the incidence and severity of work-related injuries. A main recommendation from the Committee on Injury Prevention concerned the need to develop large-scale data bases from which rates of work-related injuries could be estimated. Such data bases might serve several purposes including the derivation of basic descriptive statistics about work-related injuries, and the establishment of baseline rates of work-related injuries for use in evaluating preventive programs.

A major, unused data base which addresses the need for information about work-related burn injuries is the New England Regional Burn Program (NERBP), one of six sites participating in the National Burn Demonstration Project. The NERBP collected information concerning persons admitted to any of 240 of New England's 256 acute-care hospitals for treatment of a new burn injury. Among the types of information collected were demographic characteristics of the victim, employment status, occupation, whether or not the burn was work-related, the type of burn, and the activity (including job task) of the victims at the time of burning.

The proposed project seeks to provide a definitive analysis of work-related injuries to residents of any of the six New England states who were burned between July 1, 1978 and June 30 1979 and who were treated as hospital inpatients in any of the hospitals participating in the NERBP. Specifically, we seek to (1) assess the contribution of work, and of specific occupational groups and tasks, to the total rate of hospitalized burn injury in New England, and to provide epidemiologic bases for the development of effective control strategies for work-related burns, and (2) explore and compare known risk factors for non-work-related burn injuries, including age, race, sex, urban versus rural residence, socio-economic status, and alcohol use, for their relevance to a better understanding of risk factors and prevention techniques for work-related burns. In addition, the project will seek to evaluate the efficiency of the National Electronic Injury Surveillance System (NIOSH-CPSC) by comparing the estimates of work-related burn frequency derived from the NEISS and NERBP data systems.

An Epidemiologic Study of Injuries in Firefighters

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Traumatic Injuries
1 R01 OH02254-01A1
06/01/87 - 04/30/89
$182,554 ($182,554 Cum)

Summary

Purpose - The overall purpose of this epidemiologic research is to determine how to reduce the risk of occupational injuries among firefighters by improving our understanding of the contributing factors.

Significance - Although firefighters are known to have a high risk of morbidity and mortality from occupational injuries, few epidemiologic cohort-based studies of risk factors for injury in firefighters have been conducted. The proposed epidemiologic study will allow detailed examination of the possible causes and risk factors for injury.

Specific Aims - One of the specific aims is to describe the occurrence of injuries among Baltimore City and County fire service workers by conducting a concurrent twelve-month prospective cohort study. There are 2700 career and 3000 volunteer firefighters in the study population. About 300 disabling occupational injuries and 2000 non-disabling injuries are expected during the study period.

The second aim is to examine more specific risk factors of individual firefighters for disabling injury by doing a case-control study. Cases will be 300 incident cases of disabling injury among city and county fire service workers identified from the concurrent prospective study. One matched control will be selected for each case from among uninjured fire service workers.

Variables of interest fall into 3 categories: (1) personal attributes of fire service workers, (2) work pattern factors, and (3) situational and environmental factors. Outcome measures will include type of injury, severity of injury, disability, and associated costs. The circumstances surrounding the occurrence of injuries will be recorded. Data will be obtained from interviews, medical records, and department records.

Occupational Risks of Pesticide Exposure for Females

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Disorders of Reproduction
5 R01 OH00835-09
09/29/82 - 08/31/88
$41,526 ($358,711 Cum)

Objectives

The present project is designed to characterize some of the reproductive hazards that confront females engaged in occupations which subject them to potential exposure to pesticides. It will evaluate the effects of a known reproductive toxin on both the ovary and ovulatory ability of a non-pregnant female and on the embryological development of the ovaries of females exposed prenatally to the toxic agent. This study intends to determine how inhibition of normal reproductive activities is induced by such agents by ascertaining the effects of such chemicals on steroid hormone levels, specific follicle populations and cell surface characteristics of the different cellular components of the ovary.

Methodology

Virgin female CD-1 mice were employed in this study. Adults were randomly distributed into three treatment groups: a chlordecone-treated group, a group treated with estradiol-17B (E-17B), and sesame oil vehicle control group. Mice were exposed to these agents for two, four, six or eight weeks. Weekly procedures consisted of five consecutive daily exposures followed by two days of no treatment. This time-table was established to mimic an ordinary five-day work week which would represent the maximum weekly exposure to which a female working with such a compound, might be subjected. Chlordecone (0.062, 0.125 and 0.25 mg), estradiol-17B (0.1 mg) and sesame oil were administered in a 0.2 ml volume by oral gavage. During the final week of exposure exogenous gonadotropins were administered to determine whether the ovaries could elicit an ovulatory response to these gonadotropins. At the end of the prescribed time period, animals were sacrificed. Livers were removed from the mice and prepared for gas chromatographic analysis. Oviducts were flushed to tabulate the number of eggs ovulated in response to the gonadotropins. Ovaries were also removed to determine the number and ratio of the different follicle populations.

Prenatal studies consisted of exposing a pregnant female mouse to either chlordecone, E-17B or sesame oil between Day 6 and Day 15 of pregnancy. Animals were sacrificed at Day 18. Fetuses were weighed and sex was determined. Fetal livers were removed for gas chromatographic analysis of pesticide incorporation. Fetal ovaries were subjected to similar procedures as described above.

Progress and Accomplishments

Results obtained, thus far, in the adult mouse study reveal that chlordecone at a dosage of 0.25 mg causes a significant decrease in the ovulatory response of the ovaries following four and six week exposures when compared to both E-17B and vehicle controls. The absence of this response has been shown to be reversible within three weeks following cessation of exposure. In order to determine the reason for the lack of response of the chlordecone-exposed ovary, follicles and enclosed oocytes were tabulated in the ovary. Results revealed that there was an increase in the number of athletic large follicles following four-week exposure which meant that there was no ready pool of follicles to ovulate in response to the gonadotropins. Liver weights of chlordecone-exposed mice increased significantly and linearly from two to six weeks of exposure with a plateauing of values between 12-19% of body weight at six weeks. Similarly, incorporation of chlordecone by the liver rose dramatically at first and the leveled off at the longer exposure times.

Preliminary evidence obtained in the prenatal studies indicates that chlordecone does not induce any external malformations nor any deviation from the normal sex ratio. The female offspring displayed vaginal openings significantly earlier than those of controls. In addition, when the mothers exposed during their first pregnancy were allowed to deliver a second litter, the time of vaginal opening in this second litter was also significantly advanced.

Significance

The decreased ovulatory response seen in adult females following chlordecone exposure suggests that since there are sufficient exogenous gonadotropins available to stimulate ovulation and since ovulation is drastically impeded in chlordecone-treated animals, the effect of the pesticide might be exerted at the ovarian level. Data also reveals that even though exposure occurs during a previous pregnancy, offspring from a subsequent pregnancy are affected. Data soon to be obtained on steroid hormone levels, cell membrane alterations of different cell types in the ovary will be useful in determining where, within the ovary, such a toxic agent might have its target.

Publications

Swartz WJ: Effects of 1,1-Bis(p-chlorophenyl)-2,2,2-trichlorethane (DDT) on Gonadal Development in the Chick Embryo: A Histological and Histochemical Study. Environ Res 35:333-345, 1984

Swartz WJ: Effects of Carbaryl on Gonadal Development in the Chick Embryo. Bull Environ Contam Toxicol 34:481-485, 1984

Swartz WJ, Mattison DR: Benzo(a)pyrene Inhibits Ovulation in C57BL/6N Mice. Anat Rec 212:268-276, 1985

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Swartz WJ, Schutzmann RL: Liver Response to Extended Chlordecone Exposure. Bull Environ Contam Toxicol 39:615-621, 1987

Swartz WJ, Mattison DR: Galactose Inhibition of Ovulation in Mice. Fertility and Sterility, Vol 49, No. 3, p 522-526, 1988

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Reproduction Hazards of Dinitrotoluene Toluenediamine

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Disorders of Reproduction
5 R01 OH01520-03
12/01/83 - 11/30/86
$190,839 ($499,650 Cum)

Objectives

The general goals of this study have been the same as defined in the original application, i.e. to define the anti-fertility effects of 2, 4-DNT (DNT) and the reproductive toxicity of 2, 4-TDA (TDA) in male rats.

Methodology

Groups of adult male rats were fed varying amounts of compound in their diet for 10 weeks or were placed on normal chow for an additional 11 weeks. Fertility was tested by mating males with receptive females and determining breeding ability. The reproductive performance of male rats were evaluated by determining mating, fertility, and viability indices. (i.e. # of sperm-positive females/# of exposed females per group, # of pregnant females/# of sperm-positive females per group, and # of live fetuses/# of implantation sites). The effects of exposure to the agent were assessed further, after sacrifice of males, at 10 weeks and at 11 weeks post TDA treatment by measuring serum LH, FSH, testosterone, epididymal sperm counts, reproductive organ weights, and by examination of the cellular architecture of the seminiferous tubules.

Progress and Accomplishments

We have established that diets containing 0.03% TDA fed ad libitum to male rats for 10 weeks results in decreased mating frequency and an increase in in-fertile matings. Light microscopic examination of the testes from these animals revealed reduced numbers of sperm in the seminiferous tubules and cauda epididymides. No effect on the number of viable implants or resorptions was found among pregnancies from males exposed to this dose or to lower dose levels of TDA.

Treatment with 0.03% of TDA for 10 weeks reduced the weight of the seminal vesicles and epididymides, reduced serum testosterone levels, and decreased cauda epididymal sperm counts. The reduced epididymal sperm reserves persisted in the TDA-treated animals placed on normal diet for 11 weeks. Furthermore, serum luteinizing hormone concentrations were increased and weights of epididymides and testes were reduced in these 11 week post TDA-fed animals.

Significance

These studies demonstrate that TDA exerts a toxic effect on spermatogenesis in the male rat. The results indicate that TDA is capable of reducing fertility and of exerting an inhibitory effect on sperm production at nontoxic doses. It appears that TDA has deleterious effects on androgen production and action, but may not be capable of inducing mutagenic changes in the germinal cell of the male rat. The diminished cauda epididymal sperm counts 11 weeks after TDA treatment, suggests that the agent induced damage to the spermatogonical stem cells. The precise mechanism by which TDA exerts its adverse reproductive effects remains to be established.

Publications

Thysen B, Varma SK, Bloch E: Reproductive Toxicity of 2,4-Toluenediamine in the Rat. Effect on Male Fertility. J. Toxicology and Environmental Health, 16:763-769, 1985

Thysen B, Bloch E, Varma SK: Reproductive Toxicity of 2,4-Toluenediamine in the Rat. Spermatogenic and Hormonal Effects. J. Toxicology and Environmental Health, 16:763-769, 1985

Reproductive Effects of Leads or Solvent Exposure at Work

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Disorders of Reproduction
5 R01 OH01910-03
04/01/84 - 06/30/87
$81,657 ($194,924 Cum)

Objectives

The aim of this study is to investigate whether exposure of men and women to organic solvents or inorganic lead at work causes spontaneous abortions or malformations in the offspring.

Methodology

The exposed groups consist of women and men who, according to the biological monitoring data, have been exposed to organic solvents or lead through 1972 to 1982. The cases of spontaneous abortions will be identified from the hospital discharge register maintained at the National Board of Health. The congenital malformations in the children of the exposed women have been identified from the Finnish Register of Congenital Malformations. The figures are compared with controls who have given birth to a normal child. The controls have been selected from the workers who have ever been biologically monitored because of solvent or lead exposure.

Progress and Accomplishments

The collection of the biological measurements of solvent and lead exposure as a computerized data file with the personal identification codes has been completed. The number of solvent measurements was about 22,000 and lead measurements about 60,000.

The investigation was continued as four case-control studies: Reproductive outcome of 1) female, and 2) male workers exposed to organic solvents: 3) female, and 4) male workers exposed to lead. All women who had been treated at hospital for spontaneous abortion or who had a malformed child were defined as cases. Three age-matched controls were selected for spontaneous abortion cases and five controls for malformation cases from the women who had given birth to a normal child. Similarly, cases and controls were selected from the wives of men for each abortion case. A questionnaire has been sent to the women and to the men and their wives to confirm the exposures during the first trimester of pregnancy.

1) Among the women monitored for organic solvents 120 spontaneous abortions and 14 births of malformed children were found. The response rate was 85% for cases and controls. Exposure to organic solvents was significantly associated with spontaneous abortions (OR 2.2, p value 0.01) when adjusted for previous spontaneous abortions, parity, smoking and use of alcohol. The odds ratio was higher than unity, but insignificant for aromatic or halogenated hydrocarbons; for aliphatic hydrocarbons (includes non-aromatized mineral oil distillates as well as those with < 20% aromatic compounds, like White Spirit) the odds ratio was significantly increased (OR 2.4, p value 0.06).

2) Among the wives of men monitored for organic solvents 172 spontaneous abortions were found. The response rate for cases was slightly higher (79.1%) than for controls (72.7%). According to the preliminary results the men in the case families reported more often (84.3%) exposure to solvents during 80 days before concievment than did the men in the control families (74.0%). The case men, also, reported more often exposures to aromatic hydrocarbons than the control men. The matched pair analysis continues. There were 33 families with a malformed baby (cases) and 168 controls were selected for them. The response rate for cases was 75.8% and for controls 74.4%, total 74.6%.

3) Among the women monitored for lead exposure there were 123 spontaneous abortions. The response rate for cases was 74.0% and for controls 73.5%. The blood lead (B-Pb) was measured < 1 year before the first trimester of the pregnancy or during the pregnancy from 17 cases and 32 controls in the final study population. Only three cases and controls had B-Pb concentration > 1.4

μmol/L (> 29 μg/100 mg). In the preliminary analysis the unadjusted OR for spontaneous abortions in this high exposure group was 1.6. There was no risk in the groups with lower B-Pb. The matched pair analysis is still going on. There were 6 congenital anomalies in the material and 30 controls were selected The questionnaire was sent to 5 cases and 30 controls. The response rate for cases was 80%, for controls 67%; total 69%. The matched pair analysis is beginning soon.

4) Among the wives of the men monitored for lead there were 519 spontaneous abortions. The questionnaire was sent to 515 case families and 1,204 control families. The response rate for cases was 72.2% and for controls 74.5%. There were 51 congenital malformations in the families; questionnaires were sent to them and to 151 control families. The response rate for cases was 66.7% and for controls 76.8%. The matched pair analysis on the effects of exposure is still going on.

Significance

The result of the study - the possible effects of organic solvents or lead at various exposure levels on the pregnancy outcome of the workers - can be used in prevention of health hazards. They can also be helpful for the understanding of dose-effect relationships and for the planning of occupational hygienic standards.

Publications

Taskinen H, Sallmen M, Hemminki T, Hemminki K, Lindbolm ML, Anttila A: Exponering for Losningsmedel Enligt Biologisk Monitering (in Swedish). Abstrakt. 35. Nordiske Arbejdsmiljφmφde, 22. - 24.9. Helsingor, Danmark, 1986

Effects of 27MHz Radiation on Somatic and Germ Cells

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Disorders of Reproduction
5 R01 OH02148-03
09/27/85 - 08/31/88
$127,964 ($362,628 Cum)

Objectives

To determine thresholds and dose-response relationships for the effects of 27 MHz radiofrequency radiation (RF) on mammalian somatic and germ cells in vitro under accurately measured and precisely controlled exposure conditions. To determine the extent to which effects are dependent upon RF frequencies, cellular responses to 27 and 2450 MHz radiation are compared directly.

Methodology

Well-characterized somatic cells (erythrocytes, neutrophils, glioma [C5 and LN-71], fibroblasts, and HeLa) and germ cells (mouse ova and sperm) will be exposed under conditions of accurately measured electric and magnetic field strength in a temperature-controlled coaxial line RF cell exposure system or a temperature-controlled microwave waveguide exposure system. The dependent variables will include: 1) cell viability and morphology, 2) phagocytosis and chemotaxis, 3) plasmalemma cation permeability, 4) cell cycle regulation, 5) DNA and protein synthesis, 6) sperm viability and motility, and 7) fertilization (in vitro).

Progress and Accomplishments

Frequency dependence of cellular responses in vitro has been investigated by simultaneous exposure of cell cultures to 27 MHz or 2450 MHz CW radiation at identical SAR's under precise temperature control (37 ± 0.2°C). Exposure of human glioblastoma cells (LN-71) at 50 W/kg for 2 hr to 2450 MHz microwaves resulted in metabolic stimulation as indicated by increased uptake of labeled thymidine and uridine and mitochondrial activity at 3 and 5 days post exposure. Simultaneous exposure of the same cell line to 27 MHz RF radiation, in the same SAR range of 50 to 200 W/kg, resulted in metabolic suppression, as indicated by decreased uptake of thymidine and uridine on days 3 and 5 post exposure, with no effect on mitochondrial activity. Exposure to either frequency did not cause morphological alterations as assayed by phase contrast light microscopy. Studies of the comparative effects of these frequencies on structure and function of mouse sperm have been undertaken. Mouse spermatozoa were exposed in vitro for 1 hr at 37°C to 50 W/kg 2450 MHz radiation or 90 W/kg 27 MHz radiation. The results indicate that 2450 MHz exposure of sperm caused a statistically significant decrease in *in vitro* fertilization of mouse ova, whereas 27 MHz exposure caused a consistent but smaller, nonstatistically significant, reduction in fertilization. A highly statistically significant decrease in fertilization occurred in three experiments in which spermatozoa were exposed to 27 MHz CW radiation at an SAR of 50 W/kg.

The effect of 27 MHz radiation, at SARs of 50 to 200 W/kg, or 2450 MHz radiation at SARs of 25 to 50 W/kg, on lymphocyte mitogenesis, have been investigated by assaying ^{3}H-thymidine incorporation in PHA-stimulated human peripheral lymphocytes 3 days postexposure. Exposure to 27 MHz radiation for 2 hr results in a suppression of lymphocyte mitogenesis in all experiments conducted to date. In contrast, 2450 MHz microwave radiation appeared to increase mitogenesis at SARs of less than 50 W/kg, with evidence of decreased mitogenesis at 50 W/kg.

Human erythrocytes have been used to investigate the effects of 27 MHz and 2450 MHz radiation on cellular energy metabolism using phosphorous nuclear magnetic resonance spectroscopy (PNMRS). Comparison of PNMR spectra of erythrocytes exposed for 2 hr to 27 MHz or 2450 MHz radiation at 50 W/kg, with spectra of sham-exposed cells revealed the following:

1. The spectra of 27 MHz exposed cells exhibited: a) chemical shift of γ-ATP, b) appearance of

an upfield shoulder in the γ-ATP resonance; c) decrease in amplitude of intracellular inorganic phosphate; d) increased ATP to ADP conversion; e) no change in intracellular pH.

2. Spectral alterations in erythrocytes exposed to 2450 MHz microwave radiation included: a) small chemical shift change and relatively large decrease in amplitude of serum phospholipid resonances; b) alteration of 2,3 diphosphoglycerate (DPG) resonance peak amplitudes; and c) no detectable change in intracellular pH. These results suggest differences in the effects of 27 MHz and 2450 MHz radiation on erythrocyte energy metabolism. HeLa cells have been exposed at 37 ± 0.2°C for 2 hr to 27 MHz or 2450 MHz radiation at a SAR of 50 W/kg. 2450 MHz exposure did not alter the uptake of ^{3}H-thymidine immediately postexposure. There was, however, a significant increase in ^{3}H-uridine incorporation during exposure, followed by a decreased uptake in cells pulse labeled immediately after or 1 or 2 hrs postexposure. The experiments conducted to date involving 27 MHz radiation indicate a postexposure decrease in the uptake of ^{3}H-thymidine and ^{3}H-uridine. We do not yet have data on the effects of 27 MHz on the uptake of these precursors during exposure. Phosphorus magnetic resonance (PNMR) spectra of HeLa cells under control conditions have been determined. To obtain data of pertinence to the basic mechanisms of RF and microwave radiation effects, we have conducted studies using liposomes as a model system to determine specific effects of two radiation frequencies on membrane permeability. Large unilamellar dipalmitylphosphatidylcholine (DPPC) and dipalmitylphosphatidylglycerol (DPPG) liposomes loaded with an aqueous chemotherapeutic drug, cytosine arabinofuranoside (ARA-C) were exposed for 30 min. to 60 W/kg CW 100 MHz or 2450 MHz radiation in vitro over a temperature range of 37 to 43°C. Liposomes were exposed in HEPES buffer or in HEPES buffer supplemented with 44% by volume fetal calf serum (FCS). Characteristic phase transition responses were detected in the range of 39 to 40°C. FCS increased maximum % release of ^{3}H-ARA-C by 20% relative to HEPES suspensions. Neither frequency of electromagnetic radiation had any detectable effect on liposome permeability or the location of the phase transition in the presence or absence of FCS.

Significance

The most significant aspect of the data accumulated to date is the conclusion that 27 and 2450 MHz RF radiation can directly alter mammaliancell function in the absence of heating. If, as indicated by the results of our study, RF radiation can directly alter cell function in vivo, the induction of effects may well depend upon the instantaneous amplitude of RF fields per se, rather than average rate of RF energy absorption (SAR) or total energy absorption (SA). This is of particular significance with respect to potential adverse effects of occupational exposure to RF fields from devices such a RF heat sealers which operate intermittently at high instantaneous power. Workers are intermittently exposed to 27 MHz, and other RF frequency radiation, at instantaneous electric and magnetic field strengths of similar magnitude to these we have found to alter cell mitogenesis, DNA and RNA synthesis, energy metabolism, and sperm function (i.e., E and H field strengths of 2V/cm and 0.4 A/cm, respectively).

Another significant finding of pertinence to the question of the relative effects of continuous versus intermittent exposure to RF radiation, is the persistence of alterations in DNA and RNA synthesis we have detected in glioma (LN71) cells *in vitro*. As noted, 3-d postexposure incorporation of ^{3}H-thymidine and ^{3}H-uridine was significantly suppressed in cells exposed for 2hr to 27 MHz CW RF at SARs of 50 W/kg or greater. Uptake appears to approach control values 5-d postexposure. These results indicate RF cell recovery or repair half times on the order of a few days, which suggests cumulation of cellular alterations from intermittent RF exposures occurring, for example, on a daily basis, such as during occupational exposure.

Finally, we have obtained data indicating that specific cellular endpoints we havek investigated are sensitive to differences in electromagnetic radiation frequency. The most pronounced indication of RF frequency dependence, to date, has been effects on human erythrocyte energy metabolism. Qualitative, as well as quantitative differences in energy metabolism have been detected following exposure to 27 MHz versus 2450 MHz CW RF radiation at the same SAR (50 W/kg). The existence of frequency-dependent cellular alterations could have profound implications with respect to the formulation of safety guidelines for human exposure, which are now based upon the premise that effects are independent of radiation frequency.

Environmental/Industrial Toxicants and Testicular Injury

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Disorders of Reproduction
5 R01 OH02191-03
09/27/85 - 08/31/88
$125,444 ($357,615 Cum)

Objectives

Studies in our laboratory have resulted in a model of hexacarbon intoxication which permits dissection of biochemical and morphological events specific for testicular atrophy. In preliminary experiments, we have demonstrated alterations in microtubule assembly which occur in association with testicular atrophy induced by 2,5-hexanedione. We propose the following working hypothesis: 2,5-hexanedione reacts with tubulin lysyl residues to form pyrroles which form covalent cross links between tubulin monomers; covalently cross linked tubulin multimers change the in vitro and in vivo kinetics of microtubule assembly producing shorter and more numerous microtubules; microtubule modification is manifested as testicular injury because of the unique dependence of the testis on the cytoskeletal integrity of the Sertoli cell.

The goal of this project is to test this hypothesis of the pathogenesis of toxicant induced testicular atrophy by fulfillment of the following objectives:

1. To utilize a γ-diketone treatment protocol to specifically correlate the temporal onset and development of testicular microtubule alterations with the appearance of testicular atrophy.
2. To evaluate the reversibility of 2,5-hexanedione induced testicular atrophy in conjunction with biochemical resolution of the microtubule abnormalities.
3. To investigate the consequences for cells and tissues, particularly Sertoli cells and germ cells in the testis, of microtubule dysfunction caused by intoxication.
4. To delineate the chemistry and biochemistry of the tubulin modification which results in abnormal microtubule assembly kinetics.
5. To determine the generality of the hypothesis by testing microtubule properties in testicular atrophy induced by additional γ-diketones, the acrylamides and carbon disulfide.

Methodology

The assembly kinetics of pure rat testis tubulin from control, 2,5-hexanedione and 3,4-dimethyl-2,5-hexanedione treated animals will be compared, along with additional biochemical and morphological parameters, during a time sequence study of the induction and resolution of the testicular atrophy. Separation of Sertoli cells from germ cells combined with electron microscopy and anti-tubulin antibody techniques will be used to focus attention on the specific cell type within the testis which is injured by 2,5-hexanedione. Tubulin cross linking will be explored as the molecular mechanism for 2,5-hexanedione induced changes in microtubule assembly. Other compounds will be evaluated for inclusion within the class of testicular toxicants which act via microtubule modification.

Progress and Accomplishments

Our studies have focused on: 1) demonstration of an *in vivo* relationship between tubulin dysfunction and testicular atrophy, and 2) elucidation of the biochemical basis of the 2,5-hexanedione-induced microtubule assembly alteration. Studies of the biochemical basis for altered microtubule assembly have concentrated on the development of *in vitro* conditions capable of mimicking the *in vivo* action of 2,5-hexanedione upon microtubule assembly and a description of the assembly abnormalities. Both in vivo intoxication with 2,5-hexanedione and *in vitro* modification of pure tubulin produce qualitatively similar alterations in microtubule assembly.

Significance

The proposed studies will further the understanding of the basic mechanisms underlying toxicant induced testicular injury. The alterations in microtubule assembly kinetics induced by 2,5-hexanedione are seen as a manifestation of the cytoskeletal disruption which results in testicular atrophy. A combination of morphological and biochemical techniques will be utilized in localizing the microtubule alteration to a particular cell type within the testis and defining the chemical nature of the tubulin modification. The significance of this proposal lies in the ability to generalize from the proposed subcellular mechanism to include these agents which act in a similar fashion. Also, during the course of the project, methods will be developed which readily allow early assessment of new agents which may be testicular toxicants.

Publications

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Boekelheide K: 2,5-Hexanedione Alters Microtubule Assembly. II. Enhanced Polymerization of Crosslinked Tubulin. Toxicol Appl Pharmacol 88, 383-396, 1987

Boekelheide K, Eveleth J, Tatum AH, Winkelman JW: Microtubule Assembly Inhibition by Porphyrins and Related Compounds. Photochem Photobiol, in press

Boekelheide K: Rat Testis During 2,5-hexanedione Intoxication and Recovery. I. Dose Response and the Reversibility of Germ Cell Loss. Toxicol Appl Pharmacol, in press

Boekelheide K: Rat Testis During 2,5-hexanedione Intoxication and Recovery. II. Dynamics of Pyrrole Reactivity, Tubulin Content and Microtubule Assembly. Toxicol Appl Pharmacol, in press

Genotoxic Exposure Assessment by Simplified DNA Analysis

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Disorders of Reproduction
5 K01 OH00035-03
09/28/84 - 02/29/88
$30,563 ($94,938 Cum)

Objectives

The objective of this project is to develop a sensitive and practical test for human exposures to genotoxic agents. The test is based on observable changes in the 340 bp subsequence produced by Eco-R1 restriction of DNA obtained from peripheral leukocytes.

Methodology

DNA is extracted from human leukocytes subsequent to *in vitro* genotoxic challenge by ionizing radiation or various chemical agents. The 340 bp alphoid sequence is subjected to Eco-R1 restriction, and secondary treatment to induce breakage at damaged sites. Breakage is determined by the distribution of a labeled 340 bp probe after electrophoresis and hybridization. Alternative methods of quantifying breakage are concurrently evaluated when appropriate.

Progress and Accomplishments

1. DNA Quantification - In order that the amount of blood required for analysis be kept to a reasonably small volume, emphasis was placed on the development of reliable DNA extraction and quantification procedures suitable for the amounts of DNA available from a 5 ml blood draw. A modification of the DABA technique of Kissane and Robbins (J. Bio Chem 233, 184) was developed which produced a linear fluorometric response over the range of 50 ng to 20 μg of DNA.

2. Alkaline Lability - It was demonstrated that electrophoresis followed by cerenkov counting clearly detects the action of *in vitro* exposures of the mutagen dimethyl sulfate (DMS) on leukocytes. DNA extracted from cells exposed to 4 treatment levels was restricted, labeled with ^{32}P, made alkaline, and subjected to gel electrophoresis. Tracks were divided into three regions by excision of the 340 bp region and the activity of each region assayed by cerenkov counting. As DMS concentrations were raised from 0 to 10 mM, the fraction of the labeled genome heavier than 340 bp fell monotonically from 93% to 3%. Similar, but less pronounced effects were obtained with the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Significance

The development of a practical assay for genetic damage in the occupational setting would have a number of practical implications, the most immediate being a supplement to conventional personnel monitoring. While an assay for general genetic damage would most probably lack agent specificity, the agent is known in many routine occupational situations and in many cases of accidental exposure. Another application would be the assessment of genetic damage in exposure conditions so complex that conventional monitoring techniques cannot produce a meaningful interpretation of the situation.

Publications

Furlong NB, Marien KD, Flook B, White J: Characteristics of Site Variation Among Clones of the 340 BP Tandemly Repeated ECO-R1 Family of Human DNA. J Biochem Genet 24, p 71-78, 1986

Role of Gap Junctions in Adverse Reproductive Outcome

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Disorders of Reproduction
5 K01 OH00059-02
09/28/84 - 08/31/87
$31,482 ($94,608 Cum)

Summary

Direct intercellular communication between adjoining cells can occur among cells *in vitro* and *in vivo*, most likely through specialized channels called gap junctions. It has been suggested that gap junctional communication plays an important role in embryonic development, germ cell maturation, and parturition. Our proposed research will investigate inhibition of junctional communication as a possible mechanism of adverse reproductive outcome, and will test the validity of several short-term assays for the detection of reproductive toxins that may act by this mechanism. The first part of our research program will entail testing known and potential reproductive toxins for their ability to interrupt junctional communication using rapid *in vitro* techniques. We will employ several different cell lines in an attempt to maximize the reliability of the *in vitro* systems as predictors for the human, *in vivo* situation. These cell lines will include Chinese hamster V79, human teratocarcinoma, human uterine, and rat hepatocyte lines. The second focus of our research will be to validate inhibition of junctional communication as a mechanism of abnormal morphogenesis using hydra reaggregation as an *in vivo* development system. By differentially labelling and later mixing and reaggregating two populations of hydra cells, we hope to demonstrate that compounds which interfere with hydra reaggregation also interrupt junctional communication *in vivo*. These results will be compared to the results from cell systems to determine the reliability of the cell systems in predicting developmental toxicity. Finally, we will investigate molecular mechanisms of gap junction function. We will focus our efforts on protein phosphorylation, since this has been associated with growth factors, receptor function, and junctional communication.

Publications

Loch-Caruso R, Trosko JE: Inhibited Intercellular Communication as a Mechanistic Link Between Teratogenesis and Carcinogenesis. CRC Critical Reviews in Toxicology 16:157-183, 1985

Loch-Caruso R, Corcos IA, Trosko JE: Acetic Acid Derivatives of 2-methoxyethanol and 2-ethoxyethanol Fail to Inhibit Metabolic Cooperation. In Vitro 22(Part II):45A, 1986

Loch-Caruso R, Corcos IA, Trosko JE: Inhibition of Metabolic Cooperation by Metals. Toxicologist 6:267, 1986

Occupational Factors Associated With Biliary Atresia

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Disorders of Reproduction
5 R03 OH01827-02
09/28/84 - 08/31/87
$5,080 ($28,995 Cum)

Objectives

Empirical observation has raised suspicion that parental occupational factors may be associated with Congenital Biliary Atresia ("B.A.") in the offspring. The principal objective of this exploratory study is to determine whether parentally reported occupations, types of employment, and/or selected chemical exposures related to the pre-conceptual, gestational, and/or neonatal periods constitute significant factor(s) associated with the occurrence of B.A., or a subgroup thereof.

Additional aims of this study include determination of:

1. Associated, selected home or avocational exposures
2. Whether teratogenic associations are related to paternal or maternal exposures, or to specific combined exposures (interactions)
3. Increased incidence of other teratogenic outcomes (including spontaneous abortion) in case sibships (correlated with parental occupation for each pregnancy)
4. Associated familial occurrence of hepatitis (both Type B and others), and other specific conditions
5. Maternal health status and perinatal complications
6. Selected demographic and socioeconomic variables

Methodology

This case-control study has selected a consecutive series of Extra-hepatic B.A. cases born within the four-year span from 1982 through 1985, from the National B.A. Registry of the American Academy of Pediatrics. For improved ascertainment, all Institutions referring the Registry are being asked to review their files and to also include the newer (infant) cases as well as any earlier cases not yet registered. Desired sample size for cases is 300 to 400 or more.

Controls, matched with respective cases on sex and age (D.O.B. within one year), and on race whenever feasible, are being collaboratively selected through the files of each respective case surgeon. They will have had surgery under twelve months of age, for one of several non-congenital abdominal conditions (including inguinal hernia, intussusception, others). A second, population-based ("Normal") comparison group will soon be available per the data from the contemporary retrospective study on Congenital Heart Disease through the courtesy of Dr. Charlotte Ferenz, M.D., M.P.H. of the University of Maryland, School of Medicine. Validation of case and control diagnoses, and obtainment of selected clinical data, will come through receipt and review of selected medical histories. Parental data will be obtained through mailed, self-administered written questionnaires, separate ones for mothers and for fathers. Each questionnaire covers relevant job history, selected exposure history, hobby and other environmental history, reproductive and extended family history, and parent's personal health history.

Follow-up with non-responder parents will entail initially sending a "reminder" post-card and then, if no subsequent response, direct phone contact(s). For those families with apparent communication or literacy problems, we will offer telephone interviews. Data collected by different modes will be stratified for comparisons.

Distribution and cross-tabulations of various occupations, occupational groupings (based on similar chemical exposures), and other environmental factors will be done by case-control status, after adjustment for potential environmental, socioeconomic, and prenatal and perinatal confounders. Multiple regression analysis will be used to weight significant variables, identify significant interactions, and adjust for confounding variables. Maternal and paternal data will be analyzed separately as well as jointly, for suspected interaction effects.

Progress and Accomplishments

1. Ongoing, close collaboration with the B.A. Registry has been maintained.
2. In the Fall of 1985, correspondence (under the joint signatures of Dr. John R. Lilly, Director of the Registry, and of this P.I.) to all the Registry referral institutions/surgeons informing them of the up-coming study and requesting their collaboration. Many have already responded positively.
3. The Pre-testing Phase is presently underway and will be concluded in February. A sample of parents of Baltimore and Maryland based children (born between 1982 and 1984) with abdominal congenital anomalies has been employed and data forms are being assessed for effectiveness and clarity.
4. Direct, ongoing long-distance collaboration with the participating medical institutions will commence on 1-30-86 and continue over several months until all demographic file data is obtained.
5. Data collection will begin in March and run through the summer months, as necessary, and will include the following activities: Mailing out of the parental questionnaires, following up with "non-responder" families, requesting of medical histories from the institutions, and coding of data as it is received.

Role of Epididymal Glutathione in Chemical-Induced Germ Cell Mutations

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Disorders of Reproduction
5 R03 OH02258-02
05/01/86 - 04/30/88
$20,115 ($37,761 Cum)

Summary

The effects of altering glutathione levels in the male reproductive tract will be studied. The proposed study will attempt to establish a link between chemical-induced reduction of glutathione in the male reproductive tract with increased susceptibility of spermatozoa to chemical-induced mutations. Glutathione is an important intracellular tripeptide that participates in a number of cellular functions, one of which has been a primary interest in toxicology: the detoxification of xenobiotics. The importance of glutathione as a protective mechanism has been clearly established, within the body (e.g., liver, kidney) but little attention has been given to the reproductive tract where chronic toxicity or chemical-induced heritable changes could have serious consequences for future generations. These studies will determine whether or not a number of environmentally important toxicants are capable of lowering the glutathione levels of the male reproductive tract, and if so, whether this change will render the animal more susceptible to the action of germ cell mutagens.

The glutathione system is comprised of glutathione and several key enzymes: glutathione-S-transferases, glutathione reductase, glutathione peroxidase and gamma-glutamyl transpeptidase. The activity of these enzymes can affect tissue levels of glutathione and therefore will be studied to determine the effects of the selected toxicants on the glutathione status of the male reproductive tract. Glutathione and its related enzymes contain a number of reduced sulfhydryls, and the activity of the entire system is essentially linked to the nucleophilic nature and activity of the thiol group. Therefore, the toxicants that will be tested were chosen because they are chemicals that either 1) have a great affinity for sulfhydryl groups in biological systems or 2) the parent compound or one of its intermediate metabolites are reactive electrophiles that will react with GSH and the intermediate metabolites are reactive electrophiles that will react with GSH and the sulfhydryl groups on the enzymes of the GSH system. Germ cell mutations represent potentially one of the most serious consequences of exposure to environmental toxicants due to induction of heritable defects transmissible to progeny. The enzymatic and non-enzymatic mechanisms which afford protection to the male germinal cell will be determined.

Publications

Gandy J. Teaf, CM, Adatsi FA, James RC, Harbison RD, The Dependence of Male Reproductive Toxicity on Germ Cell Stage. In: Fujii T, Adams PM, eds. Functional Teratogenesis, Teikyo University Press, 1987

Gandy J, Buehler LA Harbison RD, The Role of Reproductive Tract Glutathione in Protecting Against Sperm Head Alkylations by Ethyl Methanesulfonate. The Pharmacologist 19 (3): 221, 1988

Male Reproductive Effects of Acrylamide

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Disorders of Reproduction
5 R03 OH02345-02
09/29/86 - 09/28/88
$22,650 ($45,161 Cum)

Objectives

1. To delineate the stage(s) of spermatogenesis in male rats most adversely affected by acrylamide.
2. To examine factors such as copulatory dysfunction-sperm transport and spermatotoxicity that may contribute to acrylamide induced preimplantation loss.
3. To determine whether acrylamide causes fertilization failure.

Methodology

Study I: Specific Stages of Spermatogenesis Affected by Acrylamide. The dominant lethal assay was chosen to determine the specific stage(s) of spermatogenic affected by acrylamide. Males were dosed subacutely with 0, 5, 15, 30, 45, or 60 mg/kg of acrylamide for 5 days by gavage. The treated males were serially mated to untreated females over one spermatogenic cycle (Weeks 1, 2, 3, 4, 7 and 10). The treated males were mated to untreated females and the products of conception were analyzed on day 15 of gestation as pre- and post- implantation loss of the embryo.

Study II: Assessment of Copulatory Function-Sperm Transport and Spermatotoxic. Copulatory function-sperm transport were assessed as one of the factors which may contribute to pre-implantation loss. Males were treated with 0 or 45 mg/kg of acrylamide. They were mated to ovariectomized, hormonally primed females and mating behavior parameters were recorded. Fifteen minutes after mating the females were sacrificed and assessed for the location (vagina or uterus) of sperm in the reproductive tract. The ejaculated sperm samples recovered from the uterus were evaluated for sperm parameters which included percent motility, sperm linearity, curvilinear and straight line velocity of sperm. These procedures were carried out at Weeks 1-4 after exposure to acrylamide.

Study III: Assessment of Fertilization Following Acrylamide Exposure. Males were dosed with 0, 15, or 45 mg/kg by gavage over 5 days. They were subsequently mated to untreated females at Weeks 1-4 after dosing with acrylamide. Females were killed approximately 8 hours after ovulation. Ova were considered fertilized if they contained the sperm head and tail or two pronuclei embedded in the ooplasm.

Progress and Accomplishments

In the first year of this project acrylamide was determined to affect sperm at Weeks 1-4 after exposure. This time period corresponds to the spermatid and spermatozoal stages of sperm development. Acrylamide did not influence sperm in the spermatocyte and spermatogonial stages (Weeks 7 and 10) at the time of exposure to this chemical. These results were based on the assessment of pre- and post-implantation loss of embryos at Day 15 of gestation. Results of particular interest were the two peaks of pre-implantation loss sustained at Weeks 1 and 3 after exposure. Post implantation loss is a precise assessment and reflects genetic damage to sperm. Pre-implantation loss, however, is much less precise and may reflect the contribution of other factors. Subsequent experiments focused on factors which may potentially contribute to pre-implantation loss. The results of these experiments demonstrated that a number of factors contribute to pre-implantation loss. Acrylamide was indicated to cause substantial copulatory dysfunction-sperm transport defects at Week 1 after exposure. In addition, acrylamide was shown to decrease percent motility and curvilinear velocity of sperm at Week 3. Finally, acrylamide significantly decreased fertilization rates in untreated females mated to treated male rats.

Significance

Acrylamide has a wide variety of applications and as a result appears in a number of environmental and occupational media. It is used in the water and waste treatment industry as a flocculating agent, in sewer grouping operations, in the paper industry as a strengthening resin, and in the production of polyacrylamide gels.

The investigations reported here provide deeper insight into the causes associated with acrylamide reproductive toxicity in the male rat. The loss in fertility noted in rats may also extend to humans. Future studies may elucidate the extent of such a relationship. The studies of pre and/or post implantation loss presented here have also provided an opportunity to examine the circumstances related to early fetal loss. Little is known about early embryo lethality (spontaneous abortion in humans) and the information obtained from these studies may provide a better understanding of this phenomenon.

Publications

Sublet VH, Smith MK, Zenick H: Acrylamide (ACR) Induced Fertilization Failure, Biology of Reproduction Supplement No. 1 Vol 36, p. 86, 1987

Sublet VH, Smith MK, Zenick H: Acrylamide (ACR) Induced Preimplantation Loss in Rats, Toxicologist, February, 1987

Sublet VH, Smith MK, Zenick H: Spermatogenic Stages Associated with Acrylamide Induced Dominant Lethality, Toxicologist, Vol, 7 No, 1, p 178, 1987

Prenatal Lead Exposure and Skeletal Growth

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Disorders of Reproduction
1 R03 OH02376-01
5/01/87 - 04/30/89
$22,650 ($22,650 Cum)

Objectives

It is hypothesized that altered calcium metabolism caused by maternal lead exposure during pregnancy will result in retarded skeletal development in fetal and weanling rats. The following aims will be addressed:

1) To determine the effect of maternal lead exposure on morphological indices of fetal skeletal development.
2) To assess the effect of maternal lead exposure on maternal and offspring calcium metabolism.
3) To quantify the effect of maternal lead exposure on rat bone modeling by bone histomorphometry.
4) To investigate the mechanism of lead effect on early bone development.

Methodology

Stage 1. Female Spraque-Dawley rats are being used, subdivided into five treatments groups of 10 to 15 rats each. The control group rats are given deionized water (0.00125% acetic acid) to drink. The lead-exposed rats are treated with 50, 250, 500 or 1000 ppm lead in 0.00125% acetic acid. Exposure begins at day 21 (weaning) and continues for 8 weeks. Food consumption, water intake, body weight gain, and tail length growth rate measurements are made every 2 to 3 days. At the end of 8 weeks of exposure, the females are mated with males of the same age and strain. After killing the rat dams at day 21 of gestation, the rat fetuses are taken by caesarean section. Fetal body weight, sex, placental weight and intrauterine position is recorded. A histological staining technique (Alizarin Red S) is used for the preparation of fetuses for skeletal morphological assessment. The lengths of all fetal tibias and femurs are measured, and the mean number of ossification centers per litter is selected bones is enumerated. Maternal lead exposure is monitored via blood lead (Pb-B) measurements by graphite furnace atomic absorption spectrophotometry (GFAAS) every 2 to 3 weeks during the 8 weeks of exposure, and on day 21 of gestation. maternal femoral calcium, phosphorus, magnesium and lead levels are measured by GFAAS. The same elements are measured in the rat fetuses and placentae. Stage 1 will be completed by January 1988.

Stage 2. The mode of administration of lead, the mating technique, numbers of animals studied, and the blood lead monitoring periodicity will be the same as described above in the Stage 1 study. In addition, ionized and total blood calcium measurements will be taken on day 11 and 18 of gestation. The offspring from each of the control and lead-exposed rat litters will be allowed to suckle for 21 days during which two sequential calcein injections in buffered saline will be administered to half of the offspring per litter at day 21 and day 23. The dams will be maintained on lead during this period. The remaining litter-mates of the labeled offspring will be injected ip with buffered saline on day 21 and day 23 post-parturition. The labeled and unlabeled offspring and their dams will be sacrificed at day 24 post-parturition. Maternal and offspring Pb-B and serum calcium (total and ionized) measurements will be taken at that time. The labeled offspring will be used for histomorphometric measurement of boe modeling. All dams and the unlabeled offspring (control and lead-exposed) will be taken for the measurement of femoral calcium and lead levels. Stage 2 will be completed by September 1988.

Stage 3. The experimental direction taken in Stage 3 will depend on the experimental outcome of the Stage 1 and Stage 2 studies. Decreased bone formation rates or increased bone resorption in lead exposed rat offspring would justify the examination of hormonal calcium metabolism regulation and bone modeling processes. Stage 3 will be completed by April 1989.

Progress and Accomplishments

Stage 1 was begun in June 1987. The results of Stage 1 will be available by January 1988. Assessment of maternal body weight gain, tail growth rate, food consumption and water intake during lead exposure prior to pregnancy has been accomplished. The results indicate that lead exposure is associated with decreased maternal rat tail length growth rate in the 1000 ppm lead treatment groups ($p = 0.0009$), and reduced body weight in the 500 and 1000 ppm lead treatment groups ($p = 0.0436$ and $p = 0.0001$, respectively). Food intake rates in the lead treatment groups are not reduced compared to the food intake rate in the control group. Water consumption is reduced in the 250, 500 and 1000 ppm lead treatment groups relative to the control group water consumption.

Significance

The results currently available suggest that lead may inhibit maternal rat body weight gain and tail length growth rate, independent of food intake. The results pertaining to the effect of maternal lead exposure on fetal skeletal ossification and body weight will be available by January 1988.

Disorders of Reproduction Among Female Veterinarians

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Disorders of Reproduction
1 R03 OH02380-01
05/01/87 - 04/30/89
$21,177 ($21,177 Cum)

Objectives

Objectives of this study are to examine health and occupational data concerning female veterinarians in order to: 1) describe reproductive disorder rates; 2) describe occupational exposures associated with veterinary practice; 3) compare observed reproductive disorder rates to relevant national health statistics; and 4) conduct an exploratory case-control study to identify associations between specific occupational exposures and specific reproductive disorders such as spontaneous abortion and fetal abnormalities.

Methodology

The proposed research project will compile and analyze data which have been collected by the investigators concerning the reproductive health and occupational exposures of the 3005 female veterinarians who graduated from U.S. veterinary colleges between 1970 and 1980, inclusive. The cohort was surveyed by a mailed, self-administered questionnaire instrument designed with input from NCHS and NIOSH. Administration of the questionnaire included multiple personalized remainders to maximize participation. For each respondent, the questionnaire elicited information on the following: history of (non-zoonotic) chronic illness, injury and accident history, reproductive history, history of zoonotic disease, rabies exposure, employment history, occupational exposures to select chemical and physical agents, and personal behaviors such as drug, alcohol, and tobacco use.

Frequency of specific reproductive events/outcomes will be calculated for age, year of graduation, practice type, and rural/urban cohorts. Descriptive statistics will be generated for occupational exposures to potential reproductive health hazards. Reproductive disorder rates will be compared to national statistics generated from data collected by the 1980 National Natality and Fetal Mortality Surveys and 1982 National Survey of Family Growth. A nested case-control study will examine associations between specific reproductive disorders and occupational exposures suggested by results of descriptive analyses.

Progress and Accomplishments

To date, questionnaire administration is complete, with the exception of non-respondent follow-up. Overall response rate is currently estimated as 82%. Questionnaire responses are now being prepared for computer storage; data editing, data reduction, and the initial phase of descriptive analyses will begin shortly. A telephone follow-up of the non-respondents is also being planned.

Significance

The proposed research will provide new information about the reproductive health, occupational exposures, and associations between occupational exposures, and reproductive disorders among female veterinarians. No such information is currently available. The number and proportion of female veterinarians has increased dramatically over the past twenty years and continues to increase. The potential occupational exposures of veterinarians include many known or suspected reproductive health hazards including anesthetic gases, antineoplastic drugs, ethylene oxide, hormones, ionizing radiation, and pesticides. The results of this research will have implications for a much larger number of women in other health and health-related occupations and women employed in agriculture.

Menstrual Function in Nurses Exposed to Cancer Drugs

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Disorders of Reproduction
1 R03 OH02383-01
09/29/87 - 09/28/89
$22,924 ($22,924 Cum)

Summary

Antineoplastic agents have been reported to have variable effects on menstrual function in patients receiving treatment for cancer. The effect of long-term, low-dose occupational exposure on menstrual function has not been studied. The goal of this research is to investigate the effects on menstrual function associated with occupational exposure to antineoplastic drugs. Specifically, the research aims to 1) assess the reliability of self-reported menstrual and reproductive histories and 2) to study differences in occupational exposure to these drugs and any associated effects on menstrual function.

A national sample of 1103 oncology nurses have agreed to participate in a follow-up study of health effects associated with exposure to antineoplastic drugs. Currently a sample of 1000 nurses nonexposed to these drugs is being recruited and it is anticipated that 800 will agree to participate in a follow-up study. The entire sample will be mailed a brief questionnaire requesting information on current employment status, antineoplastic drug exposure, and the use of protective equipment. Information on current menstrual cycles and general health will be obtained. A subsample of 200 nurses will also be asked to fill out menstrual history questions identical to those in the previous study so that test-retest reliability can be determined. Correlation analysis will be done to test the reliability of self-reported menstrual histories. Chi square analysis will be used to compare the incidence of menstrual symptoms among exposure groups. Logistic regression methods will be used to relate the presence of menstrual symptoms to background and exposure variables.

The results of this study will provide information on health risks associated with exposure to antineoplastic drugs. Positive findings will indicate the need to establish policies to protect the health of exposed workers and to investigate further the factors contributing to an increased risk of toxic effects.

Adverse Pregnancy Outcomes Among Cosmetologists

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Disorders of Reproduction
1 R03 OH02548-01
09/29/87 - 09/28/89
$21,750 ($21,750 Cum)

Summary

Cosmetology, a predominantly female employment sector, entails substantial exposure to chemicals. Adverse pregnancy outcomes have never been studied in this occupational group. The proposed study will assess whether employment in cosmetology is associated with an increased risk of spontaneous abortion, pre-term delivery or low birth weight. A set of potential hazards will be addressed, including exposure to chemical agents such as hair dyes, hair spray, permanent wave solutions, detergents, and sterilizing solutions, and physical work demands such as prolonged standing. In a retrospective cohort design, a sample of 3800 North Carolina cosmetologists licensed in 1984 will be identified through the state license register, which includes both active and inactive cosmetologists. The study will focus on their most recent pregnancies restricted to the time period 1984 to 1988. The frequency of adverse pregnancy outcomes among licensed cosmetologists who were working around the time of pregnancy will be compared to that of licensed cosmetologists who worked in other occupations or as full-time homemakers. A mailed questionnaire will elicit information on demographic background and lifetime occupational and reproductive history. Detailed information on chemical exposures, physical work demands, pregnancy outcome, and potentially confounding factors such as smoking, alcohol consumption, and previous pregnancy outcomes will be sought for the most recent pregnancy. Stratified analysis and logistic regression will be applied to assess the independent relationship between employment in cosmetology and these adverse pregnancy outcomes.

Occupational Neuropathies Due to Industrial Chemicals

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Neurotoxic Disorders
5 R01 OH00823-08
08/01/82 - 12/31/89
$157,703 ($873,634 Cum)

Objectives

The overall goal of this project is to study the molecular mechanism(s) of joint neurotoxic action of industrial chemicals. The neurotoxic action of test chemicals is being studied when applied alone or in combination. The test chemicals are n-hexane and metabolites, iso-butyl ketone, toluene, and O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN). These studies investigate the molecular effect(s) on the nervous system and on liver microsomal xenobioticmetabolizing enzymes.

Methodology

The experimental animal used in these studies is the adult hen, since it is sensitive to neurotoxicity produced by n-hexane and related chemicals and delayed neurotoxic organophosphorus compounds such as EPN. In order to simulate human conditions, dermal and inhalation treatments are used since they are the major routes of human exposure to test chemicals both in the factory and in the field. The molecular mechanisms responsible for the accumulation of neurofilaments, a pathognomonic feature of n-hexane neuropathy, are being studied. Spinal cord neurofilaments are isolated and analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using anti-neurofilament triplet protein antibodies and protein phosphorylation. Liver microsomal cytochrome P-450 isoenzymes are isolated using column chromatography and characterized by SDS-PAGE and western blots using antibodies for cytochrome P-450 isoenzymes.

Progress and Accomplishments

Initial studies in this project have established the hen as a sensitive test animal for neurotoxicity induced by n-hexane and metabolites via intraperitoneal injection, inhalation, dermal, and oral administration. The neurotoxic potency of n-hexane and metabolites, in the hen, in descending order is: 2,5-HD > 2,5-HDOH > MnBK > n-hexane. The morphology and distribution of the neuropathologic lesions of n-hexane-type chemicals were distinct from those produced by organophosphorus compounds, although both classes of chemicals produced similar clinical signs in the hen. Simultaneous daily dermal application of EPN and inhalation of MBK vapor potentiated the neurotoxic action of either neurotoxicant alone. Also, although MiBK did not produce neurotoxicity in hens, it synergized the neurotoxic effect of the weak neurotoxicant n-hexane. Additive or potentiating effect resulted from simultaneous dermal exposure of hens to EPN and n-hexane and its metabolites. This joint neurotoxic action may be related to the induction of hen hepatic cytochrome P-450 by MnBK, MiBK, and EPN. Also, enhanced dermal absorption of EPN with n-hexane, MnBK, and 2,5-HD might have been a contributing factor. Simultaneous inhalation of n-hexane and MiBK vapors resulted in neurologic dysfunction and cross-linking of the three neurofilament triplet proteins; 70-kDa, 160-kDa, and 220kDa in the spinal cord of hens. Also, these proteins exhibited a decreased Ca^{2+}-calmodulin-dependent kinase phosphorylation. These effects depended on MiBK concentration while exposure to n-hexane vapor had no effect on hen hepatic microsomal cytochrome P-450. MiBK and n-hexane vapors induced cytochrome P-450. Also, liver microsomes from hens treated with MiBK, a mixture of MiBK and n-hexane or phenobarbital produced more EPN oxon when incubated with EPN, but no effect resulted from n-hexane or B-naphthoflavone treatment. Neither treatment with EPN nor n-hexane alone or together had any effect of hepatic microsomal cytochrome P-450. On the other hand, concurrent treatment with MiBK and EPN or EPN/n-hexane significantly increased cytochrome P-450 content. These results suggest that MiBK induces some form of cytochrome P-450. Disposition, pharmacokinetics, and metabolism of a single dermal 50 mg/kg (7.5 μCi/kg) dose of $[^{14}C]$2,5-HD have been studied in hens. 2,5-HD disappeared nonexponentially from the application site with a half-life of 6 hr. After 48 hr, ^{14}C was eliminated as follows: 35%, expired as volatile materials (mostly 2,5-HD), 15% in excreta, and 12% as $^{14}CO_2$. The half-life for elimination of ^{14}C was longest for muscle (71 hr) and shortest for adipose tissue (12 hr). 2,5-HD was metabolized to 5-hydroxy-2-hexane and 2,5-dimethylfuran.

Significance

The studies on the joint neurotoxic action of organophosphorus insecticides and industrial solvents in the hen have demonstrated: a) although both classes of chemicals produce similar clinical signs, the morphology and distribution of their neuropathologic lesions are distinct, b) simultaneous dermal application of EPN and inhalation of MBK vapor potentiated their neurotoxic effects, c) daily dermal application of EPN in combination with n-hexane, MnBK, 2,5-HD, or 2,5-HDOH resulted in a potentiating or additive neurotoxic action of EPN, and d) MiBK synergized the neurotoxicity of n-hexane. n-Hexane is metabolized to 2,5-HD which causes the accumulation of neurofilaments as the result of a) decreasing the Ca^{2+}-calmodulin dependent Kinase phosphorylation of neurofilament triplet proteins, b) cross-linking of these proteins, and c) diminishing the breakdown of cross-linked neurofilaments.

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Chronobiology and Occupational Health Hazards

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Neurotoxic Disorders
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$119,699 ($846,369 Cum)

Summary

The long-term objective of this study has been to determine what role metabolic rhythms have in influencing response to toxicity from physical stimuli, industrial toxins and therapeutic agents. Among those that have been, or currently are being investigated include: irradiation, paraquat, urethane, malathion, mercuric chloride, insulin, glucagon, epidermal growth factor (EGF), somatostatin, ACTH, and gastrin. The toxicity response in mice to all has been shown to be circasian-stage dependent. Moreover, some of the above polypeptides stimulate RNA and DNA synthesis in a number of tissues including the alimentary canal (EGF, insulin and gastrin) whereas others are predominantely inhibitory (glucagon, somastatin, and ACTH), again all responses have been found to be circadian-stage dependent. The aim of the present application is to continue such studies, but also, to determine if the chronobiological findings already gained can be used to reduce overall toxicity.

The model chosen has been cell proliferation in certain regions of the intestinal tract, which also undergoes remarkable circadian variation. Can the fact that fasting, beginning at a certain circadian stage, for a span of 36 hours or less reduce the fraction of proliferating cells and thus be used to protect the gut from damage brought about by: (1) a physical agent such as irradiation which affects the mitotic spindle or (2) a chemical agent such as cytosine arabinoside, which specifically interferes with DNA synthesis? Moreover, will any of the above mentioned peptides, when given before, simultaneously, or subsequent to either a physical or chemical induced injury, protect different regions of the gut from damage or enhance repair? Another objective is to gain insight into the mechanism of action of EGF induced cell membrane protein phosphorylation in the gut of normal adult mice and those treated with irradiation.

These studies have relevance for industrial toxicology, gastroenterology, immunology, and endocrinology.

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Molecular Mechanisms of Diketone Neurotoxicity

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Neurotoxic Disorders
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$46,290 ($133,853 Cum)

Objectives

A number of industrial and commercially-available chemicals cause a toxic neuropathy in experimental animals and man characterized by abnormal accumulation of neurofilaments within vulnerable axons. This group includes the important solvents n-hexane and methyl n-butyl ketone, both of which are metabolized to 2,5-hexanedione (2,5-HD). This γ-diketone is believed to directly interact with axonal proteins to induce the observed effects.

The present investigation examines the hypothesis that γ-diketones react with lysine ϵ-amine groups of neurofilament (NF) and other axonal cytoskeletal proteins to form hydrophobic 2,5-dialkylpyrrole adducts. It is further postulated that pyrrole adduct formation is the critical initiating event in γ- diketone neuropathy, resulting in disruption of the specialized cytoskeletal protein transport mechanism within the axon and accumulation of neurofilaments.

Methodology

Techniques designed to investigate the mechanism of pyrrole formation in a model protein (bovine serum albumin; BSA) include in vitro exposure to 2,5-HD, [^{14}C]-2,5-HD, or perdeuterio ([D^{10}])-2,5-HD. Analysis for pyrrole content and lysine binding is performed by colorimetric assay, liquid scintillation counting, and quantitative amino acid analysis. Protein conformational changes are determined by protease cleavage with or without prior reduction of disulfide linkages and treatment with denaturants.

The comparative neurotoxicity of γ-diketone isomers is assessed in rats following daily ip injection with equimolar amounts of appropriate compounds. Clinical and neuropathological evaluations are performed, in addition to biochemical and pharmacokinetic studies. Pyrrole adduct formation is determined in non-eural and in axonal cytoskeletal protein, and pyrrolylated proteins are analyzed by gel electrophoresis.

Progress and Accomplishments

The quantitative relationships between pyrrole formation and total covalent binding were examined in BSA treated with [^{14}C]-2,5-HD. These studies revealed that lysine was the only amino acid modified by the diketone. In addition, it was demonstrated that 2,5-HD was capable of reacting with previously-formed pyrroles to yield higher adducts. Pyrrolylation was accompanied by substantial conformational changes in BSA, such that the hydrophobic adducts tended to be less accessible than the parent lysine functions.

The neurotoxic and pyrrole-forming potentials of 2,5-HD were compared with those of [D^{10}]-2,5-HD in the rat. Due to a requirement for C-H bond breaking in the reaction mechanism, the latter derivative was expected to exhibit a primary isotope effect, thus forming the pyrrole at a slower rate. Adult, male Wistar rats were administered either 2,5-HD or [D^{10}]-2,5-HD. At termination, animals administered 2,5-HD exhibited a significantly greater body weight loss than those given [D^{10}]-2,5-HD. Moderate to severe hindlimb paralysis was present in the 2,5-HD groups while only mild effects were seen in [D^{10}]-2,5-HD-dosed rats. Neuropathological changes were prominent in spinal cord from 2,5-HD-treated animals, while no effects were present in rats given the deuterated derivative. Pyrrole adduct concentrations in serum and axonal cytoskeletal proteins from 2,5-HD-treated animals were two- to three-fold higher than in rats given equimolar doses of [D^{10}]-2,5-HD. Tissue concentrations of each diketone isomer were not significantly different, indicating similar uptake of native and deuterated 2,5-HD.

Significance

Our studies have provided further insight into the mechanism of action of the neurotoxic γ-diketones. The findings suggest that pyrrolylation of NF protein *in vivo* is likely to result in conformational changes which may have profound effects upon the function of these important structural elements. In addition, the results support an absolute requirement for pyrrole formation in γ-diketone neurotoxicity. It is anticipated that findings derived from this project will ultimately lead to assessment of the axonal cytoskeleton as a potential target site for these and other occupational and industrial neurotoxins.

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Mechanisms of Occupational Neuropathies

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Neurotoxic Disorders
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Objectives

The overall goal of this project is to study the mechanism(s) of organophosphorus compound-induced delayed neurotoxicity (OPIDN) in a sensitive mammalian species, i.e., the cat in comparison with the test animal to study OPIDN, i.e., the chicken. Initial studies in this project have suggested that organophosphorus compounds may bind directly to a calcium/calmodulin activated protein kinase (CaM Kinase II). Subsequent conformational changes lead to increased Ca^{2+}-calmodulin dependent ATP phosphorylation of its substrate proteins, i.e., tubulin, neurofilament proteins, and MAP-2. It is proposed that these effects alter the normal interactions of these cytoskeletal proteins and lead to eventual degeneration of the axon. Increased ATP phosphorylation of tubulin and MAP-2 results in aggregated insoluble filamentous polymers distinct from microtubules. Also, increased the activity of CaM Kinase II leads to increased intracellular concentration of Ca^{2+}, which may result in enhanced Ca^{2+}-activated proteolytic destruction of neurofilaments. Accumulation or dissolution of cytoskeletal elements leads to disruption of axoplasmic transport and accumulation of mitochondria at the distal portion of the axon. Disrupted mitochondria release their Ca^{2+} stores into axoplasm. This overloads and disrupts any still functional axonal membrane control mechanisms for intracellular/extracellular ionic gradients, which leads to focal internodal swelling and Ca^{2+}-dependent proteolysis, followed by focal degeneration that spreads somatofugally to involve the entire distal axon.

Methodology

The present studies into the mechanisms of OPIDN are carried out in the hen, the experimental animal for OPIDN, and the cat, a mammalian species that may be a better model for extrapolation to humans. Test chemicals used in these studies are the delayed neurotoxic organophosphorus compounds tri-o-cresyl phosphate (TOCP). Parathion is used in some experiments as a non-delayed neurotoxic compound (negative control). CaM kinase II, α- and β-tubulin, MAP-2, and neurofilament triplet proteins are isolated. In vitro phosphorylation of cytoskeletal proteins is performed using $[\gamma\text{-}^{32}]$ATP followed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Identification of cytoskeletal proteins is carried out using one- and two-dimensional SDS-PAGE, peptide mapping, and immuno blotting.

Progress and Accomplishments

A preliminary dose-effect study has characterized delayed neurotoxicity induced by dermal application of a single or daily doses of TOCP in the cat. Initial phase of this project characterized neurotoxic esterase (NTE) as a membrane bound protein, has a molecular weight of 155-178 kDa, its target size determined by radiation inactivation is 105 kDa, and its axonal transport rate is 150 mm/day. While these results failed to explain the role of NTE in the pathogenesis of OPIDN, other results suggested that delayed neurotoxic organophosphorus compounds phosphorylate a target protein, i.e., CaM Kinase II which interferes with its normal function in phosphorylating cytoskeletal proteins. TOCP in vivo and DEP in vitro increased CaM Kinase activity which resulted in enhanced phosphorylation of a- and B-tubulin, MAP-2, and neurofilament triplet protein. These changes correlated with the following characteristics of OPIDN: test chemical, whether delayed neurotoxic or not, dose-dependence, and time course of the effect; and animal sex sensitivity, age selectivity, and species susceptibility.

Significance

Based on the results of this project and on previous studies, our hypothesis for the mechanisms of OPIDN may be stated as follows:

1. Delayed neurotoxic organophosphorus compounds phosphorylate a neurotoxic target protein, e.g., CaM Kinase II, resulting in conformational changes of the enzyme which increased its enzymatic activity.
2. Increased activity of CaM Kinase II leads to an increased CaM Kinase II-dependent phosphorylation of cytoskeletal elements, i.e., tubulin, MAP-2, and neurofilament triplet proteins.
3. Enhanced CaM Kinase II phosphorylation of cytoskeletal proteins leads to their loss of capacity to assemble into polymers, instead they aggregate into solid masses and/or their Ca^{2+}-activated proteolysis.
4. As a consequence, axonal transport of cytoskeletal protein is impaired which causes accumulation of mitochondria at the distal portion of the axon.
5. Mitochondria would then break down and release their Ca^{2+} which promote Ca^{2+}-dependent proteolysis and disrupt intracellular/extracellular ionic gradients. This leads to focal swelling followed by focal degeneration that spreads somatofugally to involve the entire distal axon.

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Acrylamide Neurotoxicity: Roles of Oxidative Metabolism

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Objectives

We are testing the hypothesis that acrylamide (ACR) monomer, a neurotoxic chemical which produces a central-peripheral distal axonopathy, inhibits enzymes of oxidative metabolism causing a decrease in production of high energy phosphates. More specifically:

1. Does ACR selectively inhibit the activity of oxidative enzymes in neural tissue?
2. Does ACR inhibition of glycolytic and/or oxidative enzymes produce a decrease in high energy phosphate production by mitochondria or the concentration of ATP and CP in nerve?
3. Is there a correlation between the reduction in axoplasmic transport caused by acrylamide and a reduction of high energy phosphate levels in peripheral nerve?

Methodology

Quantitative histochemical assays for nicotinamide-adenine dinucleotide tetrazolium reductase (NADH-TR) activity in specific motoneurons, DRG neurons, Purkinje neurons, hepatocytes, parietal cells and renal tubule cells as well as in vitro and in vivo biochemical assays of lipoamide dehydrogenase (LpDH) and cytochrome c reductase (CCR) on a variety of organ homogenates and glial and neuron-enriched fractions of cerebral cortex were used to determine the neural specificity and degree of inhibition by ACR. The ability of neural mitochondria to produce ATP from pyruvate after exposure to ACR was also measured. The effect of a single injection of ACR upon axoplasmic transport of radiolabeled protein in the sciatic nerve was compared with the level of ATP and CP in the nerves (luciferin-luciferase bioluminescence assay) at the same time points of measurement.

Progress and Accomplishments

ACR was shown to decrease the NADH-TR activity of motoneurons by 17-20% after 5 and 10 days of exposure. A single injection of 50 mg/kg ACR produced a 33% inhibition in motoneuron NADH-TR activity within one hour of injection; the activity returned to normal after 3 days. A dose-dependent inhibition from 1-10mM ACR with a maximal inhibition of 54% was found. MbACR was less effective in producing the *in vitro* and *in vivo* inhibitions. ACR was shown to produce neuron-specific inhibition of NADH-TR activity while MoACr produced a significant inhibition in Purkinje neurons only. The degree of inhibition was found to be inversely related to levels of glutathione. CCR activity was neuron-specifically inhibited by ACR *in vivo* and *in vitro* whenever neural and non-neural tissues were compared. LpDH did not show any neural specificity of inhibition. Neither LpDH or CCR activity was specifically inhibited in neurons as compared to glial enzymes. We concluded that although certain neuron specific enzyme inhibitions were observed, the small degree of inhibition and the lack of neural-specificity in several cases, eliminated these enzymes as critical sites of action of ACR. It remained possible that other oxidative enzymes were significantly and specifically inhibited by ACR. Therefore, the effects of ACR on the rate of formation of ATP in brain mitochondria were determined. Preincubation of brain mitochondria with 0.73 mM ACR (equivalent to a single daily exposure) for 30 minutes at 37°C did not significantly alter the rate of formation of ATP. In addition, the exposure did not change the concentration of ATP within the mitochondrial fraction. Other neurotoxicants (IDPN and DMHD) did not significantly alter mitochondrial ATP production; 2,5-HD did produce a significant change.

The rate and capacity of axoplasmic transport of radiolabelled proteins down the sciatic nerves were significantly, and in a dose-dependent manner, reduced by 50-100 mg/kg ACR. The quantity of protein transported was affected to a larger degree than the rate. This observation has lead us to postulate that the neurotoxicants which produce distal dying-back degeneration of nerves do so by a reduction in the transport of critical macromolecules required to support the distal axon. However, at the time frame of axoplasmic transport changes the levels of ATP and CP in the contralateral sciatic nerves or in the nerves of other animals were not significantly decreased.

Significance

Although ACR is capable of significant inhibitions of oxidative enzymes, these inhibitions appear inadequate to effect a reduction in the ability of the mitochondrion to produce ATP. The reduction in the quantity of protein transported in the nerve is severely reduced by a single exposure to ACR but this change is not caused by a reduction in high energy phosphates. Other modes of action are under consideration.

Publications

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Mechanisms of Occupational Distal Axonopathy

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Neurotoxic Disorders
5 R01 OH02065-03
05/01/85 - 04/30/88
$191,861 ($471,054 Cum)

Objectives

These studies are designed to further understanding of how neurotoxic agents alter normal neuronal processes to produce axonopathy. Central-peripheral distal axonopathy is produced in laboratory animals repeatedly dosed with acrylamide and is a useful model of neuronal degenerative disease. Acrylamide neurotoxicity may be initiated by an inhibition of the retrograde axonal transport system which interrupts the flow of information from axon to perikaryon. As a consequence, the neuron fails to mount the appropriate repair responses when challenged distally and the axon undergoes distal-proximal degeneration. To test this novel hypothesis we are measuring markers of repair-associated gene expression following axotomy in the presence and absence of systemic acrylamide intoxication.

Methodology

The bipolar sensory neurons of mouse dorsal root ganglia and their projections located in the sciatic nerve constitute the *in vivo* model system employed to study perikaryon repair responsiveness. Experimental protocols are designed to study the inhibitory effects of acrylamide and other agents including colchicine and vinblastine on the increases in perikaryon ornithinedecarboxylase (ODC) activity (an index of repair response) following transection of the sciatic nerve.

Progress and Accomplishments

Recent progress includes the development of assay methods to measure ODC activity of mouse dorsal root ganglia. Data have demonstrated that following transection of the sciatic nerve, ODC polymerase activities are significantly elevated in dorsal root ganglia by day 2 post-axotomy. Experiments with several dosing regimens of acrylamide reveal that this agent can attenuate the increase in ODC activity following axotomy. When taken in concert with earlier experiments which demonstrated that single and repeated doses of acrylamide cause a reduction in the rate of retrograde axoplasmic transport, these results provide strong support for our hypothesis of altered repair responsiveness in acrylamide-induced neurotoxicity.

Significance

Monomeric acrylamide is an environmental and occupational neurotoxin which causes polyneuropathy in humans. Despite many years of intensive research, the exact toxic mechanism of acrylamide remains unknown. Diminished perikaryal repair capacity, in the presence of low-level axon damage, represents a novel mechanism by which toxic agents may alter neuronal function or structure. Our studies suggest that primary axon degeneration in acrylamide-induced (and perhaps with other axonal toxins) neuropathy may result from a cascade of pathophysiological events: a) acrylamide (or another axonal toxin) impairs retrograde transport; b) this attenuates the putative retrogradely transported signal to the perikaryon; c) this results in a perikaryal response insufficient to restore the integrity of the entire axon; d) the resupply of required materials via anterograde transport reaches the proximal but not the distal axon, a situation that may be further aggravated by defective anterograde transport; e) the axon consequently undergoes distal-to-proximal degenertion.

Publications

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Moretto A, Sabri MI: Progressive Deficits in Retrograde Axon Transport Precede Degeneration of Motor Axons in Acrylamide Neuropathy. Brain Res, in press

Validation of a Neurobehavioral Test Battery

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Neurotoxic Disorders
7 R01 OH02384-01
09/27/85 - 10/31/87
$159,618 ($317,405 Cum)

Objectives

To validate a computer-administered neurobehavioral test system for use in field situations for detecting the intoxicating effects of solvents.

Methodology

Transient changes in central nervous system (CNS) function were assessed using a computer-administered neurobehavioral evaluation system (NES). Industrial hygiene sampling, exhaled breath analysis, and urine analysis were coupled with biomathematical dose models to estimate CNS concentrations of solvent. To reduce the complexity of exposure estimation, fiberglass boat-builders were studied. They were exposed almost exclusively to styrene. NES testing was performed at the beginning of work shift, after peak-exposure activity near mid-day and at the end of work shift. Data analysis relates changes in behavioral performance to environmental styrene levels, styrene in breath, styrene in urine and estimated brain dose of styrene.

Progress and Accomplishments

Work in the first year concentrated on identification and recruitment of styrene-exposed workers, development of breath sampling and analysis methods, and data collection on one-half of the subjects. Data collection and laboratory analyses of environmental samples were completed in the second year. Completion of data analysis and report writing are currently underway.

Significance

This study should demonstrate the utility of the NES in assessing intoxicating effects of solvents in field study situations and provide information directly relevant to the setting of workplace exposure standards. It should improve our ability to monitor exposure and effect and to establish dose-effective relationships in solvent-exposed workers.

Publications

Letz R: Occupational Screening for Neurotoxicity: Automated Techniques. Accepted for publication in Toxicology October, 1987

Peripheral Nervous Effects of Workplace Neurotoxins

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Neurotoxic Disorders
7 K01 OH00064-03
09/28/84 - 08/31/87
$32,400 ($97,200 Cum)

Objectives

To develop methods that are accurate, sensitive, and efficient for field evaluation of peripheral nervous system functions in workers exposed to neurotoxic agents.

Methodology

Three aspects of peripheral nervous system function have been selected for study: Vibration sensitivity, temperature sensitivity, and tremor. The project consists of five phases: (1) examination and modification of test apparatus and data collection procedures, (2) reproducibility testing (3) normative data development (4) testing of exposed populations and (5) development of protocol manual.

Progress and Accomplishments

Vibration and temperature sensitivity: Instruments from Sensortek, Inc., were selected. We implemented procedures for estimating sensory thresholds that are more time efficient than the one proposed by the instrument manufacturer. We have performed computer simulations comparing our procedure, the manufacturer's suggested protocol, and other standard procedures.

We have collected data using a computer-assisted protocol on more than 110 unexposed workers for development of norms for both the temperature and vibration test devices. In addition, we have collected sensory test data during field studies of solvent exposed painters. 113 painters were tested with the temperature device and 55 were tested with the vibration device. Exposure-effect analyses on these data have been completed. Data collection for reliability and stability of these exposure effects in painters have been submitted for publication. Manuals presenting the protocols developed are being completed.

For tremor testing, peripheral equipment has been assembled and computer software integrated to allow acquisition and storage of tremor signals as well as performing Fast Fourier Transforms of signals for frequency analysis. Reliability of the method in field testing situations was not found to be suitable, and further development was discontinued.

Significance

These tests and new procedures should expand our ability to study the effects on the PNS of neurotoxic agents in the workplace.

Publications

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Bove FJ, Letz R, Baker EL: Sensory Thresholds Among Construction Trade Painters. A Cross-Sectional Study Using New Methods for Measuring Temperature and Vibration Sensitivity, in press

Test for Neurotoxins Using Caenorhabditis Elegans

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Neurotoxic Disorders
5 R03 OH02093-02
06/01/85 - 05/31/87
$14,748 ($31,689 Cum)

Objectives

The overall aim of the project is to develop a screening test for neurotoxins using the nematode Caenorhabditis elegans. The test organisms are exposed to selected chemicals and changes in behavior are evaluated using computer tracking.

Methodology

A strain of E. coli is grown on experimental plates until a lawn is well formed and then 0.4 mL of an aqueous solution of the test chemical is added to the plates. The plates are desiccated for 24-hours at 50% relative humidity to allow for the excess water to evaporate. A number of three to four day-old C. elegans are placed on each plate. After 24-hours two types of evaluations are made: lethality and computer tracking to determine behavioral changes. Control cultures are tested concurrently with the neurotoxins.

With lethality tests, death is determined by the total lack of movement and/or response to probing with a needle. For behavioral studies, the worms are removed from the plates, washed, and placed on 1% agar. The worms are positioned under a video camera that is interfaced to a microcomputer. This arrangement allows for the simultaneous tracking of several hundred nematodes. The movements of the animals are analyzed to determine both the rate of movement and the number of changes in directions (reversals). In the future, responses to sensory stimulation will also be measured.

Progress and Accomplishments

Lethality studies have been completed for seven metals: Hg, Cu, Pb, Cd, Zn, Al, and Ni. For each metal a dose-response has been determined and the LC50 has been calculated using both probits and logits.

Computer tracking experiments have been conducted on Hg and to a lesser extent on Cu. The nematodes exposed to Hg show a biphasic response - at very low Hg concentrations there is hyperactivity that appears to peak at exposure concentrations of about 7 ppm Hg and as the concentration increases the activity falls to well below the controls. This peak activity Hg concentration is approximately 10% the nematode LC50 of 60 ppm Hg. The computer tracking for Cu has only shown decreased activity as the Cu concentration increases; however, very low concentrations of Cu have not yet been studied.

Significance

The lethality data has been compared to the LD50 data from mammalian studies and, except for of one metal (Cd), there is a good correlation between the results. Although much more data is needed, it is believed that this test species shows much promise as a basic model for range-finding studies and initial lethality screening tests.

The computer tracking studies are in a preliminary state, but the hyperactivity observed with Hg supports our hypothesis that computer tracking of C. elegans can provide a rapid, inexpensive means of screening chemicals for neurotoxicity.

The Effects of Impulse Noise on the Auditory System

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Noise-Induced Hearing Loss
7 R01 OH01152-08
07/01/80 - 09/28/89
$163,440 ($932,166 Cum)

Objectives

The purpose of this research is to understand the relation between the parameters of impulse noise (peak pressure, duration, number, repetition rate, exposure duration, etc.) and the effects on hearing as assessed by anatomical, physiological, and psychophysical techniques in the chinchilla.

Methodology

Several mechanical or electrical-mechanical devices are used to produce realistic noise impacts. Hearing is tested, the animal is exposed to impulse/impact and its hearing is tested for the following forty days. Routine data collection consists of measurements of hearing sensitivity, auditory discrimination and cochlear histology. More detailed studies of certain experimental groups will include scanning E.M., as well as more discriminating psychoacoustic measures of hearing.

Progress and Accomplishments

This project has led to a number of new insights into the effects of exposure to impulse noise. We have provided data to support the hypothesized relation between the duration of an impulse and its hazard to hearing; we have shown (morphologically) that impulse noise can damage the cochlea by direct mechanical destruction. This mechanical damage is different than the cochlear damage following continuous noise and is probably responsible for different audiological symptoms produced by impulse and continuous noise. Several years ago we showed that impulse and continuous noise can synergistically interact. We have documented that noise induces a number of the changes in the neural code leaving the ear and have related these changes to the symptoms of noise-induced hearing loss. Recently, we have finished a large, parametric study of the validity of the Equal Energy Hypothesis.

Significance

The results have direct implications for noise standards. Heretofore, we have either neglected or underestimated the traumatic potential of impulse noise. Our research shows the peculiarities of impulse noise and suggests several situations where impulse noise increases the audiological hazard.

Publications

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Functional Correlates of Cochlear Injury

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Noise-Induced Hearing Loss
5 R01 OH02128-04
09/01/84 - 08/31/89
$129,148 ($479,104 Cum)

Objectives

The major goal of this project is to determine with behavioral, physiological, and anatomical studies how the magnitude, pattern, and growth of hearing loss and structural damage are altered as the parameters of noise exposure are varied. Secondary objectives include evaluating hearing loss and cochlear damage as a function of age in a group of chinchillas that have never been exposed to noise. In addition, acoustic measures of spontaneous otacoustic emissions are being made from the ear canals of all noise-exposed chinchillas. Finally, field studies in industrial workers are being conducted to determine the effect of nonoccupational noise exposure on hearing levels of individuals exposed to moderate to high levels of noise at work.

Methodology

Hearing thresholds are obtained by behavioral methods in chinchillas before, during and after noise exposure. Physiological measures are then obtained in some animals, and all animals are prepared for microscopic evaluation of the cochlea. Acoustic measures are made with a small probe microphone positioned in the ear canal of the anesthetized animal. Industrial surveys are made by audiometric database analysis of thresholds obtained as part of the company hearing conservation program.

Progress and Accomplishments

Progress has been made in all the areas originally proposed and the project has expanded to include studies of aging and evaluation of industrial databases. Important findings to date include the following: 1) Under some schedules of intermittent exposure, hearing thresholds recover by as much as 30 dB even though the noise exposure continues. The locus of the recovery phenomenon is at the periphery, probably at the level of the hair cell; 2) Evaluation of hearing after long (>3 yrs) exposure to continuous noise shows that ATS is constant and that PTS has not reached the level of ATS even for these long exposures; 3) Old chinchillas do not show behavioral presbycusis even though there are age-related changes in the cochlea; 4) SOAEs in chinchillas are not correlated with audiogram microstructure but are associated with regions of hearing loss and cochlear damage, indicating that SOAE production is probably a pathological process; 5) For individuals exposed to occupational noise at levels greater than 90 dBA, hunting or target shooting cause about 16 dB more hearing loss in the high frequencies than occupational exposure to noise.

Significance

The behavioral and anatomical studies of long-duration noise exposure elucidate relations between TTS, ATS, PTS and damage to the cochlea. Studies of SOAEs in noise-exposed animals provide a unique opportunity to relate SOAEs and cochlear histopathology, which will lead to a better understanding of active cochlear biomechanics. Industrial surveys help identify the factors which eventually cause occupational noise-induced hearing loss in millions of Americans.

Publications

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Clark WW, Solomonson M: Spontaneous Otoacoustic Emission from a Chinchilla Ear Following Exposure to Noise. J Acoust Soc Am 82, S117, 1987

Hearing Hazard Associated with Industrial Noise Exposure

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Noise-Induced Hearing Loss
2 R01 OH02317-03
08/01/84 - 07/31/90
$179,307 ($511,750 Cum)

Objectives

The objective of this grant is to systematically examine the synergistic effects of noise exposure and vibration on hearing. Several different types of noise exposure (four band-limited noises of equal energy, and eight impulsive noises with different levels) and two examples of whole-body vibration at different frequency and magnitude are being combined in various exposure paradigms to assess the factors that influence the interaction between vibration and various categories of noise.

Methodology

A series of 14 control groups and 20 experimental groups has been exposed to continuous or impulsive noise, vibration or a combination of noises or vibration and noise. The exposures utilize two experimental paradigms: acute exposures, and those that produce an asymptotic threshold shift. Hearing thresholds and masked thresholds (i.e., tuning curves) are obtained before exposure and over a thirty-day postexposure period using auditory evoked potentials. The final format of the data includes a comprehensive description of the exposure, a profile of the animals hearing ability before and after the treatment, and a detailed, quantitative morphological analysis of the cochlea. The morphological analysis relies primarily upon surface preparation of the organ of Corti and the vascular tissues of the lateral wall of the cochlea and includes scanning and transmission electron microscopy of interesting or unusual pathologies.

Progress and Accomplishments

I. The Interaction between Continuous and Impulse Noise: Frequency Effects. This set of experiments illustrates some of the conditions that must be satisfied for a synergistic interaction between impulse and continuous noise to occur. The data illustrates the effect of the energy frequency spectrum of the impulse on the production of a synergistic interaction. Chinchillas were used as the experimental animal. Experimental groups containing 5 animals were exposed to one of the following noise conditions. (1) Two different octave bands of noise for one hour; 0.5-1.0 kHz at 100 dB SPL or 2.0-4.0 kHz at 95 dB SPL. (2) Impulse noise generated by an electrical spark discharge. The duration of the first positive overpressure of the Friedlander wave thus generated was varied in four steps from approximately 30 μ sec. to 80 μ sec. at a peak SPL of 158 dB. A Fourier analysis indicated that the energy peak of the impulse shifted from approximately 8 kHz down to 4 kHz. The impulses were presented at the rate of 1/min for 50 min. (3) The third series of exposures consisted of combinations of the above two sets of exposures. This experimental design allowed us to study the effect of a shifting impulse spectrum on the hearing loss acquired from very brief duration impulses. The results indicated that a synergistic interaction is most likely to occur when there is a spectral overlap of energy between the impulse and the continuous noise. Using a completely different experimental protocol and a more realistic set of stimuli, essentially, similar results were obtained in the experiments described below. In addition this second set of experiments showed that the equal energy hypothesis is of little predictive value when complex exposure paradigms are encountered.

II. The interaction of impulse and continuous noise: Energy and spectral considerations in the production of hearing loss. Realistic industrial noise environments containing impulsive and continuous noise were modeled using a 5-day exposure paradigm which produces an asymptotic threshold shift (ATS). Pre- and postexposure measures of hearing thresholds were obtained on 96 chinchillas using evoked auditory responses (EAR). Six control groups were exposed to octave bands of noise at 0.5, 2.0, and 4.0 kHz at 95, 90, and 86 dB SPL respectively or impacts of 113, 119 or 125 dB peak SPL presented once per 1, 4 or 16 seconds, respectively. Nine interaction groups were exposed to combinations of an impulse and continuous noise. The greatest spectral overlap of energy occurs between the impulse and the 0.5 kHz octave band of noise. Although each of the different impulse noise exposures were balanced to produce an equal energy exposure, an exacerbation of hearing loss was produced in animals exposed to the 119 and 125 dB impacts in combination with the low frequency (0.5 kHz) continuous noise. The audiometric and histological data are in agreement in showing that the synergistic effect gradually disappears when the spectral overlap between noises is reduced.

III. Noise and vibration interactions: Ten different exposure paradigms are being used to assess the effects of 20 Hz and 30 Hz (3g rms) vibration on susceptibility of experimental animals to noise trauma. These experiments are currently in progress and discussion of results at the time is premature.

Significance

Synergistic interactions among various ototraumatic agents have been shown to be of importance in determining the hearing loss in a number of industrial situations. The interactions between continuous and impulse noise and vibration and noise (both continuous and impulsive) are being studied using an animal model. The rationale for these studies is that continuous noise rarely exists as a sole hazard to hearing in most industrial and military work environments. Thus, exposure criteria which are based upon data obtained from experiments involving only the use of continuous noise may not be protective.

Publications

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Noise-Induced Hearing Loss and High Blood Pressure

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Noise-Induced Hearing Loss
5 K01 OH00042-03
09/28/84 - 08/31/87
$32,400 ($96,120 Cum)

Objectives

The goals of our present study are: 1) further validation of our previous noise and blood pressure study (Talbott, Amer Jour Epid 121:4, 501-514, 1985) by its replication in a group of men 55-68 who have long histories of occupational noise exposure, 2) to carry out further more detailed characterization of the audiometric profile of these occupationally noise-exposed men, and 3) administration of the revised form of the Hearing Performance Inventory as developed and tested by Lamb et. al. (Journal of Ear and Hearing, 1983). This is being used to assess the communicative difficulty of noise-exposed individuals in a variety of everyday listening situations. In addition, an index of social support is being administered. This was one of the variables found to correlate with B/P in our previous study.

Methodology

A cross-sectional design was selected in order to test the hypothesis and study the relationships already described. The men are retired metal assembly workers aged 55-68. There are 616 active members in the retirement organization. The criteria for eligibility are that the individuals be white male hourly workers between the ages of 55-685 and must have 20 or more years of exposure on the job. The average ambient noise level in this plant is > 89 dBA. We have chosen this group because: 1) they represent the most exposed segment of the plant population, 2) the previous study indicated a strong effect for severe noise-induced hearing loss and blood pressure in this group, 3) we hope for a high response rate (to rule out self-selection bias) and this group is more accessible. Union records were used to obtain the worker's name, address, department where he worked and seniority date.

In order to assure sufficient group size for stratified cells and noise-induced hearing loss comparisons, a total sample of 250 men should be sufficient.

The clinical exam included measures of height, weight, pulse and blood pressure, as well as an indepth medical and personal habits history including alcohol consumption and smoking patterns. There was also an occupational history, military and noisy hobby questionnaire, hearing performance inventory and social support scale. This exam was being administered at the Union Hall in the morning.

Because large variations in blood pressure have been shown to exist within a given subject, it is difficult to detect differences between groups and may lead to misclassification of individuals. Therefore, multiple blood pressure measurements using standard procedures have been implemented in this investigation. Blood pressure was determined three times within five minutes after a ten minute rest period and then repeated by a second staff fifteen minutes later.

Standardized audiometric testing was supervised by a Ph.D. audiologist who possesses a Certificate of Clinical Competence in audiology from the American Speech and Hearing Association. Audiometric tests included were: 1) pure tone air conduction; 2) pure tone conduction; 3) speech reception threshold, and 4) speech discrimination scores in quiet and in competing noise, and the Hearing Performance Inventory.

Progress and Accomplishments

There were 616 men who were members of the union retirement organization as of April 1, 1984. They made up our study population from which we obtained potential participants. Of these, 137 were ineligible because they had moved out of state or were beyond the 50 mile radius of our study area. All people who had ever worked at the plant automatically become members of the retirement organization. There should be little selection bias with regard to membership. Of the 479 remaining, 110 were ineligible because they were over or under age or currently working. Sixty-eight was chosen as the cut-off because at this age, presbycusis (effect of aging on the acoustic mechanism) may confound the effects of noise exposure. Fifteen people from the original sample were deceased, 67 have refused and 245 were screened. An additional 42 were unable to be contacted. Our overall response rate was 78.6%.

Data analysis will be complete in mid December 1987. The average age of the participants was 63.0 (3.2). Body mass index was directly related to blood pressure. The distribution of length of employment indicates an average of 29.9 years spent in this plant. There is significant hearing loss in this population particularly in the high frequencies, with an average mean decibel loss of 55, 62 and 61 at 3, 4 and 6 kHz. There is some recovery evidenced at 6000 and 8000 Hz. The basic groups for comparison in this study will be men 56-63 and 64-68 with severe noise induced hearing loss versus those without severe noise-induced-hearing loss. We are defining severe noise induced-hearing loss as greater than or equal to 65 dB loss at 3, 4, or 6 kHz (± 20 dB) bilateral. The prevalence of hypertension (greater than 90 mm diastolic or currently taking BP meds) will be determined for noise induced loss groups as well as for those with and without hearing impairment (low frequency loss).

It is also hypothesized that the more objective measures of speech discrimination (a measure of word distortion) and speech reception (threshold or responses) will correlate with the hearing performance inventory (an index of lifestyle interference). This was developed by Stanford H. Lamb at Stanford and is being used for this study.

Significance

The results of our previous study suggest that severe noise-induced hearing loss and blood pressure levels are associated and both may be due to long-term noise exposure among susceptible individuals. An alternate hypothesis may be that in the older worker, noise exposure in addition to the aging process may lead to poor speech discrimination, discomfort to loud sounds, etc., such that it may result in reduced social interaction and interference in lifestyle and increased stress which may affect BP levels. This study is designed to address this question. Preliminary results indicate a greater proportion of the older retired workers (age 64-68) with severe noise-induced hearing loss were currently on blood pressure medication. Mean unadjusted blood pressure for these 2 groups were 146.1 mm Hg and 140.4 mm Hg systolic and mean diastolic 85.5 and 81.0 ($p < .05$) respectively.

Publications

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Matthews KA, Cottington E, Talbott E, Kuller LH, Siegel J: Stressful Work Conditions and Diastolic Blood Pressure Among Blue Collar Factory Workers. Amer J of Ep 126, No 2, pp 280-290, Aug 1987

Auditory Temporal Acuity Following Noise Exposure

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Noise-Induced Hearing Loss
5 R03 OH02141-02
09/27/85 - 08/31/87
$21,426 ($40,910 Cum)

Objectives

The purpose of the study is to describe subtle, temporary changes in human temporal acuity brought about by listening to moderately intense noise for a brief period of time. The effect of signal frequency, signal level, and recovery time on gap detection threshold will be examined following the noise exposures.

Methodology

Octave-band noises with a brief temporal gap will be used as the experimental stimuli. Off-frequency spectral cues will be minimized with a broad-band masker. An adaptive two-interval forced-choice psychophysical paradigm will be used to measure gap detection threshold (GDT). The GDT's will be obtained both before and after a 5-minute, octave-band noise exposure. Simulus generation and control of the experiments will be accomplished with a laboratory microcomputer.

Progress and Accomplishments

To date, the accomplishments related to the project include: (1) selection and purchase of a microcomputer and related laboratory apparatus, (2) equipment set-up, and (3) development of computer software to generate the stimuli and control the experiments.

Significance

The results may provide the basis for developing a more sensitive tool for evaluating the effects of noise on hearing. Information from this project may also help us understand the temporal processing capabilities of some hearing-impaired individuals.

Audio Mechanical Transducer Communication Aystem

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Noise-Induced Hearing Loss
1 R43 OH02313-01A1
09/01/87 - 03/31/88
$50,000 ($50,000 Cum)

Objectives

The Audio Mechanical Transducer (AMT) Communication System will significantly improve the environment of workers exposed to high ambient noise. The communication system requires the use of existing standard hearing protection to operate. This characteristic will enhance the effectiveness of hearing protection programs. The AMT Communication system will be low cost, permit multiple audio inputs and demonstrate man/machine interface capabilities. Use of the AMT Communication system, will have a positive impact on the issue of noise induced loss of hearing and worker safety.

In addition to the technical development of the system, there are numerous safety and health issues that must be addressed. These include: the effectiveness of the hearing protector has not been degraded, the sound levels produced by the AMT are not excessive, and the system does not encumber or endanger the worker by having protrusions (antennas) or exposed wires.

Phase Two will expand on these areas and also examine the ramifications of using a communication system in high noise environments. The perceptions and needs of both management and workers must be understood for the system to be accepted and effective. Speech intelligibility research and testing will be included in an effort to establish a measurement standard for the communication system.

Methodology

Phase One of the SBIR is being used to examine the basics of the AMT Communication System. The technical questions include identifying interface requirements and the effects on frequency response of epoxy encapsulating the AMT. Protocols for evaluating the AMT - hearing protector combination are being developed in Phase One. During Phase Two, the safety evaluation of the system will be performed and site testing will begin. A research matrix that includes technical (audiology and acoustic), safety, health, management and labor will be developed. This research group will help answer design questions, fine tune system goals and provide a project feedback mechanism.

Progress and Accomplishments

Most Phase One objectives have been met. A few are still in the process of completion. The project is within the time framework established by the benchmark chart. The Test and design facilities at Camtech Inc. have been effective in developing an epoxy encapsulated AMT that has excellent frequency response and audio energy transfer characteristics. Mr. Sam Lybarger, consultant to the project, has been very helpful in equivalent circuit descriptions, AMT testing methods and identifying Phase Two technical questions. Ball State University, Department of Speech Pathology and Hearing, is currently developing the first level of safety protocols that will be used in Phase Two.

Significance

There are approximately 2.9 million individuals in the United States working in a high noise environment. Lack of communication can cause feelings of isolation and reduce the effectiveness of hearing protection programs. The inability to clearly hear warning signals has led to injuries and deaths. The majority of workers in high noise environments do not benefit from existing communication systems. The AMT Communication System addresses each of these areas.

Hearing protection programs have traditionally been based on the fear persuasion of "wear hearing protection or you will go deaf." The AMT Communication System will present an alternative, "wear hearing protection and enhance your work environment."

A Personal Dosimeter for Exposure to Ultraviolet Light

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Dermatologic Conditions
5 R01 OH02021-03
02/01/84 - 01/30/87
$87,649 ($276,594 Cum)

Objectives

There are uniformly ignored federal standards for near (315-400 nm) and far (200-315 nm) UV radiation. The standards are written with a flat response curve in the near UV and a steep curve in the far UV. The purpose of this study is to provide simple, low-cost, easily-analyzed, and interference-free dosimeters which meet the requirements of the standard and would be acceptable to the individual wearer for occupational, recreational, or therapeutic exposure to potentially carcinogenic ultraviolet light. The major hypothesis is that photochemistry of some appropriately chosen molecules could provide the required dosimeters by measuring the ratio of product to reactant.

Methodology

For the 200-315 nm region, a tube containing 0.25% 2-Butanone (MEK) in oxygen-free water is worn in an exposed place. The ethane and ethylene produced by UV photolysis are then analyzed after the end of the shift or after suspected exposure, using a gas chromatographic technique. Dilute benzal acetone in isopropanol is one candidate for the 315-400 nm range, with the product analyzed simply by UV absorption or comparative colorimetry.

The far UV photolysis is carried out in quartz tubes whereas the near UV photolysis uses pyrex tubing, thus eliminating any far UV phontons. The molecules chosen provide a very close match to the required spectral shape for the radiation exposure standards, particularly in terms of the sharp cutoff at long wavelengths. This eliminates any possible interference from abundant long wavelength photons.

Progress and Accomplishments

We have demonstrated that MEK photolysis and analysis of the ethane and ethylene can be used as a dosimeter for far UV radiation and have built and demonstrated a prototype dosimeter package. We encountered unexpected containment difficulties in that the original gas phase mixtures tried were not stable in their containers for a long period for useful dosimetry. The approach to eliminate this difficulty is to use a water solution of one of the relevant molecules since much larger absolute numbers of molecules can easily be placed in the same container, and GC sampling is simplified.

Significance

The original hypothesis has been proven to be correct. The unexpected containment problem has been solved for the far UV, and a very useful protective device is the outcome. The device was first demonstrated informally at the NIOSH meeting in Cincinnati, November 1986.

Immunotoxicology of Phenols on Epidermal Immune Cells

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Dermatologic Conditions
2 R01 OH02091-03
04/01/85 - 07/31/90
$96,630 ($141,667 Cum)

Summary

A large number of antioxidants of preservatives used in industrial manufacturing, pharmaceuticals and food industries include the so-called nucleophilic (para-substituted phenols-PSP). Some of these chemicals such as monobenzyl either of hydroquinone (MBEH) and paratert butylcatechol (PTBC) are known melanocytotoxic compounds and can lead to widespread depigmentation following prolonged exposure. However, these compounds can have more complicated effects on the skin. PSP applied topically to the skin induces proliferation of keratinocytes (KC) and pigment cells. Of greater significance, these compounds markedly alter, i.e., increase or decrease, the numerical density of Ia+ epidermal Langerhans cell (LC) populations depending on the dose of phenol applied. It appears that the quantity of Ia antigen present on LC membranes may regulate the intensity of immunologic vigor in contact hypersensitivity (CHS) experiments. More ubiquitous phenols such as butylated hydroxytoluene (BHT) and 4-hydroxyanisole (4-OH anisole) when topically applied also increase the density of Ia+ LC. In addition, they increase the density of another newly discovered epidermal immune cell in the skin, so-called Thyl+ cell (EC). Thyl+ EC are bone marrow derived heterogenous populations of cells that can express a variety of membrane markers in addition to Thyl. Thyl+ EC do not appear to be T or B cells, macrophages, or melanocytes. The function of the Thyl+ EC is not known, but most likely they are part of the cutaneous immune system and function either as suppressor cells or natural killer cells. Topical applications of PSP like 4-OH anisole induce the expression of both Ia and Thyl markers on KC membranes. The mechanism for this phenomenon is unknown. From work we have completed, we concur that one function of the Thyl+ EC may be that of an epidermal suppressor cell which "down regulates" allergic contact dermatitis reactions by directly or indirectly opposing Ia+ epidermal cells. The major objectives of this proposal include: 1) to study the immunotoxicological effects of PSP on the function of the Thyl+ EC; and 2) to determine the interrelation of Ia/Thyl markers on epidermal cells following PSP exposure and its possible significance in cutaneous immune reactions. Highly enriched epidermal cells obtained by fluorescent activating cell sorting (FACS) will be placed in culture and treated with a variety of PSP compounds. Cells will then be subjected to double-labeled immuno-electron microscopy using ferritin and gold electron dense markers to simultaneously visualize Ia and Thyl markers. Semi-allogeneic skin grafts will be performed to determine if the Thyl/Ia expression on keratinocytes is due to a passive shedding from nearby LC or Thyl EC. To examine for Thyl function in particular, enriched LC and Thyl EC will be co-cultured together to determine if the Thyl cells can suppress LC presentation of antigen to syngeneic T cells as well as suppress LC activation of allogeneic T lymphocytes using a mixed lymphocyte reaction. Completion of these studies will provide new insights into the understanding of epidermal immune regulation and perhaps new approaches for prevention of or treatment of a variety of occupational allergic dermatoses.

Publications

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Cutaneous Exposure: Predictive Pathways

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Dermatologic Conditions
5 K01 OH00017-03
09/28/84 - 12/31/87
$31,174 ($93,049 Cum)

Objectives

The ultimate goal of this research is to attain the ability to predict the detrimental toxic effects of hazardous chemical exposure via the skin. Specifically, our objective is the quantitative estimation of the toxicokinetics of occupationally-encountered molecules absorbed across human skin *in vivo*. The aims may be summarized as:

a. Establish a literature database on percutaneous absorption (PA).
b. Explore pathways by which a prediction of PA kinetics may be made.
c. Develop, refine and apply new predictive models for cutaneous toxicity.
d. Assess the value of different pathways in estimating multiple exposure risks.
e. Review critically *in vivo* animal models and *in vitro* (excised skin) techniques for measuring skin penetration and to determine whether straightforward relationships exist between these systems and the human *in vivo* situation.

The long-term focus of the project is to respond affirmatively to the question: "Can the health hazard from dermal exposure to toxic chemicals be predicted correctly on the basis of fundamental biological and physicochemical principles?"

Methodology

The approach may be summarized in three components:

1. The establishment of a comprehensive percutaneous absorption database, cross referenced to physicochemical information on the chemicals studied. The review, collation, and filing for future access: a. human and animal *in vivo* results, b. *in vitro* penetration data through excised skin, and c. proposed pharmacokinetic and diffusional descriptions of skin transport and biodisposition.
2. The development of interpretive models for percutaneous absorption; pharmacokinetic and diffusional and structure-activity relationships are being investigated and refined. The models incorporate both established cutaneous biology and recognized physicochemical interactions between the penetrant and skin.
3. The assessment (and initiation) of experimental approaches to transdermal absorption; the identification of optimal methodologies for future determinations of potential cutaneous toxicity.

Progress and Accomplishments

Activity in three areas may be identified: a. Literature search and database establishment. A comprehensive evaluation of the percutaneous absorption literature has been made and database are being set up on both Apple IIe and IIc microcomputers and on the NIH Prophet resource. Appropriate software has been obtained and accessed. The Prophet system will ultimately allow merging of the percutaneous absorption information with corresponding physicochemical data on the penetrants. The subsequent aim is to utilize developed structure-activity analysis software to define quantitative relationships applicable to dermal transport.

b. Theoretical development of models for the prediction of skin penetration kinetics. Specific descriptions of the chemical input function at the skin surface have been investigated. Zero-order and first-order kinetics have been considered and the approach has been applied specifically to a few very active drug molecules (e.g. nitroglycerin, clonidine), for which well-characterized input functions are available. Good agreement between *in vivo* data and predictions based upon pharmacokinetic and physicochemical information has been obtained. Although initial progress has targeted transdermal drug delivery as the major application of the analysis, the principles involved are readily extrapolated to occupational exposure situations.

c. Exploration, extension and application of new and existing methodologies for percutaneous absorption assessment. 1. Measurements have been made of the effect of aging on percutaneous absorption in man. *In vivo* skin penetration studies reveal that there are differences in absorption between elderly subjects and young "controls" and that the differences are greatest for more water-soluble compounds. 2. Multiple-dose experiments have been performed to evaluate the predictive potential of simpler acute exposure measurements. Good correlation between penetration, surface recovery and penetrant petitioning characteristics has been obtained. 3. Structure penetration relationships for a series of nicotinic acid esters have been derived in model, *in vivo* and *in vitro* percutaneous absorption studies. Extension of the work with a range of phenolic derivatives is in progress.

Significance

Skin disease, initiated in the work place, currently represents a major occupational health problem in the United States. Prediction of the detrimental toxic effects of hazardous chemical exposure is difficult, however, because of the complexity of the percutaneous absorption process and a lack of any consistently identifiable relationship(s) between transport rate and chemical properties. In addition, the very diverse approaches, which have been used to measure skin penetration, further complicate the problem since the extrapolation of results to the human *in vivo* situation is an exercise requiring often quite unreasonable and, hence, potentially dangerous assumptions. The ultimate goal of the research proposed here is to address this area of occupational health concern. Therefore, our specific aim is acquisition of the ability to predict accurately the toxicokinetics of occupationally-encountered molecules absorbed across human skin *in vivo*.

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Occupational Health Hazards in Skin Injury by Mitomycin C

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Dermatologic Conditions
5 R03 OH01990-02
04/01/85 - 03/31/87
$10,642 ($31,446 Cum)

Objectives

The study of factors controlling the generation of cutaneous intradermal injuries from the antitumor agent MMC. This drug is a health hazard for occupation related injuries in health care workers, and treatment related injuries in cancer patients. Current and projected usage of MMC show a substantial potential risk of cutaneous injury and present treatment methods are unsatisfactory. A murine model will be used, and factors of injury generation will be studied: dose effects, injury generation time, healing time, and drug activation rate. Treatment measures that will reduce, prevent, or eliminate, cutaneous injury from the use of MMC will be studied. Since heat has been reported to potentiate the in vitro antitumor cytotoxicity of MMC, it is hypothesized that antidotal attempts with heat will worsen ulcers, and heat and cooling antidotal effects will be studied.

MMC lesions appear to resemble adriamycin cutaneous lesions. Since adriamycin induced cutaneous lesions have been reported to respond to DMSO treatment, it is hypothesized that DMSO will be effective against MMC induced cutaneous lesions. Furthermore, the reports of DMSO's effectiveness, when applied distally to the ulcer site, will be evaluated in MMC induced ulcers.

Methodology

Skin Toxicity Studies: Dehaired mice were given topical MMC intradermal (ID injections at various doses, and assessed for cutaneous toxicity. Topical antidotal applications of sodium chloride, sodium thiosulfate, N-acetylcysteine, catalase, heparin, hyaluronidase, hydrocortisone, superoxide dismutase, diphenhydramine, fumaric acid, lidocaine, isoproterenol, alpha- tocopherol, cysteine, and DMSO, were made following ID exposure and evaluated for efficacy against MMC. Because of the marked antidotal activity observed with DMSO, time dose-dependent studies were pursued to determine the optimal DMSO application schedule, and the effects of delayed DMSO application. Daily ulcer measurements were integrated to give area under the toxicity x time curve in cm^2 days, and appropriate statistics (e.g., ANOVA) performed.

Progress and Accomplishments

Skin Toxicity Studies: MMC produced dose-dependent toxicity. Ulcers appeared within 1-5 days following injection, with induration and erythema 20-fold and 10-fold respectively greater in size than the central ulcer. Erythema tended to peak by day 5, and usually resolved over a 2-4 week period. Induration also peaked at day 5, but required up to 7 weeks to completely resolve. MMC ulcers had a central necrotic appearance and resembled adriamycin induced ulcers. MMC did not produce uniformly larger ulcers with larger doses, but larger doses did require longer healing times.

Several potential antidotes significantly increased MMC ulceration. These included: N-acetylcysteine; diphenhydramine; lidocaine; and both enzymatic treatments - catalase and superoxide dismutase. Sodium thiosulfate reduced MMC ulcers, but the level of antidotal activity did not reach statistical significance. Other antidotes were ineffective at reducing MMC ulceration. These include: hyaluronidase; hydrocortisone; fumaric acid; heparin; several sulfur nucleophiles; isoproterenol; and vitamin E. Neither heating nor cooling of the skin reduced MMC ulcers.

The same antidotal pattern was evident with a higher dose of MMC. No antidotal activity was seen for most of the numerous ID pharmacologic agents evaluated. This parallels exactly, the response patterns seen with the lower dose ID MMC experiments. The *in vivo* results for heating of MMC ulcers are consistent with the *in vitro* cytotoxicity observations showing synergism between heat and MMC. Prolonged heating markedly increases the peak ulcer area from ID MMC.

In contrast, the nonpolar solvent, DMSO, applied once after ID MMC, provided complete protection against MMC ulceration following the low dose. More frequent topical applications of DMSO were detrimental and some ulceration was produced after low dose exposure. For the high dose MMC, a single topical application of DMSO immediately after MMC, was again the most effective way of reducing MMC-induced skin ulcers. More frequent DMSO applications were also detrimental against the high dose injections. There was no evidence for systemic antidotal DMSO activity. DMSO antidotal activity was still observed 4 hours after ID MMC, but did not produce statistically significant reduction of toxicity. Delayed application of DMSO for more than 4 hours after MMC completely eliminated any antidotal activity.

Significance

Reliably produced MMC ulcers in mice have been demonstrated, and the characteristics of healing profiled. A large number of potential MMC antidotes were found to be inactive. Although sodium thiosulfate showed some antidotal activity, the magnitude of the benefit is smaller than that observed for DMSO. The agents that produced no benefit can not be recommended as possible clinical antidotal treatments. Furthermore, an additional number of agents increased MMC toxicity, and should be specifically avoided in clinical settings where MMC cutaneous toxicity has occurred.

DMSO appears to give substantial protection against MMC-induced skin ulceration in the mouse when applied immediately after exposure and proximal to the ID site. The results of the DMSO treatments show that some antidotal activity exists even when given up to 4 hours after MMC ID exposure. This agent may prove to be efficacious in clinical settings with further study.

Mechanisms of Cytotoxicity by Chlorodinitrobenzene, A Potent Sensitizer

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Dermatologic Conditions
1 R03 OH02433-01
09/28/87 - 09/28/89
$26,490 ($26,490 Cum)

Summary

1-Chloro-2,4-dinitrobenzene (CDNB), a halogenated nitro derivative of hydrocarbon, is a potent skin sensitizer causing severe allergic contact dermatitis, and thus is a documented occupational hazard as well. Previous studies from this laboratory have shown that CDNB induces microtubule (MT) disassembly in mouse 3T3 cells. Blocking of certain -SH groups of tubulin, the principal protein component of MT, with -SH reagents inhibits tubulin polymerization in vitro. Since glutathione (GSH is the predominant non-protein thiol in the cell, it is reasonable to expect that GSH is involved in the dynamics of MT state. Furthermore, GSH is crucial for cellular defense against insults by toxic electrophiles by forming appropriate conjugates with the toxicants through the action of glutathione-S-transferase (GST). However, the mechanisms by which CDNB induces cytoskeletal perturbation and its relationship to the ultimate cellular injury are not clear. Therefore, the specific aims of the project are: 1) to determine whether CDNB acts directly on the cytoskeleton by binding to tubulin/MT in the cells resulting in MT disassembly; 2) to investigate the role of total cellular GSH content, which can be manipulated by the use of specific agents to either increase or decrease, in modulating the dynamics of MT assembly and disassembly and microfilament (MF) distribution during CDNB exposure; and 3) to quantitate the amount of cellular free vs polymerized tubulin and actin (the major protein of MF) by developing an ELISA assay for each protein. Studies on the relationship between the cellular GSH contents and the dynamic changes in the cytoskeleton of human fibroblasts may lead to a better understanding of the mechanisms of induction of allergic contact dermatitis caused by CDNB and other related halogenated nitro derivatives of hydrocarbon.

Occupational Stress and Health of Women LPN'S and LSW'S

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Psychological Disorders
5 R01 OH01968-03
12/01/84 - 11/30/88
$228,376 ($593,269 Cum)

Objectives

The short-term longitudinal interview study of women who are licensed practical nurses (n = 156) and licensed social workers (n = 248) focuses on the relationship between occupational stress and health-related outcomes in women. Building on the investigators' prior research, the study extends our understanding of occupational based psychosocial stress to include not only job conditions and personality traits but also: (a) occupancy of non-workplace roles; (b) the quality of experience in occupational and in non-workplace roles. Quality of experience refers to the balance between rewarding and distressing attributes or a role and is measure by scales developed previously by the investigators.

Two categories of outcomes are examined: (a) physical health indices, including both self-reports and blood pressure measurements; and (b) mental health indices, including role strain, anger, and anxiety.

Major hypotheses concern the effects on physical health and psychological distress of job conditions, occupancy of non-workplace roles, and quality of roles. In addition, possible moderators of the relationship between stressors and health outcomes are examined, e.g., the interactions of work and family roles, and personality variables such as trait anger and anxiety.

Methodology

A stratified sample (race, parental status, partnership status) of LPN's and LSW's, ages 25 to 55, who live within specified zip codes (an approximately 25-mile radius of Boston) was drawn randomly from the professional registries of the two occupations. By enlisting the cooperation of relevant groups and publicizing the study widely, we have obtained an excellent response rate of 96.6%. In individual interviews a variety of measures -- both standard scales and scales developed for this study -- assess role quality (the balance between rewarding and distressing attributes of a role), psychological distress, and physical health, (including blood pressure). During the four years of the project, data will be gathered at three points in time, one year apart. A 10% random sample of subjects was reinterviewed to establish the test-retest reliability of certain scales, e.g., job conditions, rewards, and concerns.

Casual analytic techniques, including LISREL, will be used to evaluate specific hypotheses concerning the effects on health and psychological distress of job conditions, occupancy of non-workplace roles, and quality of experience in roles. In addition, we will examine the interactions of occupational and family role variables as these may moderate the relationship between occupational stress and health outcomes.

Progress and Accomplishments

(a) Sampling: As part of the process of identifying and drawing a sample, demographic studies were conducted of women LPN's and LSW's that resulted in a decision to stratify the sample by race, parental status, and partnership status. In this way, the potential confounding of such variables as race and physical health will be avoided. Moreover, because of the low proportion of black women among LSW's, we have developed a census of all black social workers in our geographical area; we need to identify and include all such women to insure adequate numbers for analyses.

Because of unforeseen difficulties with the LPN registry, we were unable to locate the targeted number of women. The major problems were: (1) LPN's move frequently and the registry is out of date, making it extremely difficult to locate subjects; (2) the "partnered with no children" cell is very rare for LPN's since by age 25 most married LPN's have children; and (3) the actual number of existing LPN's was much lower than the estimates provided to us.

(b) Development of Measures: Major investment was made in developing measures of job conditions, social ties, physical health, rewards and concerns of various roles, and costs and benefits of multiple roles. For example, based on research currently in progress by other investigators on women's physical health, prior measures of physical health were modified to include previously neglected stress-related symptoms of women such as urinary tract problems and menstrual difficulties. Further analyses have been carried out of the rewards and concerns measures previously developed by the principal investigators in order to refine them.

In exploring procedures for determining blood pressure and accessing health records, the decision was made to collect data on blood pressure from all S's and to omit accessing of health records. Because subjects are not being identified by worksite, and because they vary greatly in their life situation, preliminary studies showed that too much uncertainty and diversity existed with respect to obtaining adequate health records. Other problems arose because of the variety of health professionals consulted, faulty memory for details, etc. Interviewers were certified by Red Cross instructors in obtaining blood pressure data; due attention is being paid to issues of reliability and validity.

Several new measures were developed and pilot tested for the second wave. Most importantly we included measures of role change concerning each major social role, i.e., paid worker, partner, mother and daughter. For each change, we ask subjects to indicate: (1) the degree to which the change was expected; (2) how much control they felt they had over it; and (3) how positive or negative it was.

We also included a measure of sex-role attitudes, items about leisure activities and friendships and about the rewards and concerns associated with aging, and open-ended questions about the role of grandmother and volunteer. Several of these areas were suggested to us by subjects, who have taken an unusual interest in the progress of the study.

Significance

Results of the first wave are being analyzed.

Impact of Automation on Industrial Inspector Stress

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Psychological Disorders
5 R01 OH02024-03
09/28/84 - 12/31/87
$108,627 ($287,927 Cum)

Objectives

The objective of this research is to determine how both human stress and human performance vary with the level of automation in an industrial inspection job. Industry is demanding higher quality of its workers and of its inspectors who are charged with maintaining and monitoring that quality. At the same time, sophisticated automation is becoming available to extend the human inspector's capabilities on some tasks within the inspection job. Inspection is known to be a stressful job and, with these two trends, a job which is likely to increase in stressfulness.

Methodology

An industrial inspection device is to be used to provide varying levels of automation. The Color Video Comparator (CVC) superimposes television images of the inspected item and a standard item in rapid succession onto a single color monitor. Any defects appear as changes in visual pattern between the successive images. In cooperation with AT&T Technologies, Inc., a stimulus set of life-sized photographs of a printed circuit board has been obtained. These photographs are of either a perfect board or one containing up to two of four different defects. Unemployed industrial workers are trained on this task, both using unaided visual inspection (the control condition) and at one of six levels of automation on the CVC:

1. Freely moving CVC to search board.
2. CVC moves in steps across board.
3. CVC moves in pre-determined step sequence.
4. Paced predetermined sequence, equal times for all areas.
5. Pattern recognition algorithm to automate search.

Performance measures are search time, stopping time and error rate. Stress measures are visual stress (Critical Flicker Fusion Frequency), postural stress (self-report scales and measures of ancilliary behaviors) and information processing load (heart rate variability, GSR). In the final year, AT&T Technologies is cooperating in replicating parts of the experiment using actual circuit board inspectors to give measures of job stress as well as task stress.

Progress and Accomplishments

The printed circuit boards, CVC and subject pool were obtained in Year 1 and the experiment at the first level of automation was run and analyzed. It was found that automation level 1 was slower than free inspection for stopping time ($p < 0.01$), but slightly better for defect detection ($0.05 < p < 0.07$ for three defects). Stress measures were not significantly different for this level of automation.

As a result of this experiment and analysis, the data collection has been expanded (GSR, Blood pressure) and shortened (2 vs 3 days). All levels of automation are being run again in year 2 with the new measures for consistency. Liaison is being maintained with AT&T Technologies for the final in-plant studies.

Significance

The results of the project will be of value to designers of automated and semi-automated systems as they will help to specify the level of automation which gives the best balance system performance and operator stress.

Publications

Drury CG, Kleiner BM: A Comparison of Blink Aided and Manual Inspection Using Laboratory and Plant Subjects, Proc of 29th Human Factors Society Annual Meeting, San Antonio, pp. 670-674, 1984

Jobs and Family Stress and Women's Work Performance

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Psychological Disorders
1 R01 OH02162-01A1
02/01/87 - 01/31/88
$118,781 ($118,781 Cum)

Summary

The purpose of this investigation is to increase knowledge of the roles family and work factors play in working woman's job performance, as measured abuse. The results will be useful for planning prevention efforts aimed at providing those women at risk with skills to adopt functional rather than dysfunctional coping behaviors. We propose a process model which suggests that work and family environments are related to certain repertoiries of behaviors in a manner reflecting individual differences. We will test the relationship of risk factors and substance use by measuring 1) workplace stress factors, 2) family stress factors, 3) personal factors, and 4) dysfunctional coping behaviors, as a basis for possible prevention and intervention strategies. To do this, we will recruit and survey, with permission and support of the AFL-CIO Appalachian Council, a sample of 650 working women, including a 20% minority subsample, on a variety of scales measuring the four categories of variable. In addition, a 5% subsample will be interviewed in groups of 4-5 by a project associate to validate the accuracy of the self reports by the total sample. The subjects will be union examine relationships among multiple measures of each variable to arrive at variables of work stress/support, family stress/support and mediating dysfunctional coping. Cluster and discriminant analyses will be performed to environments and particular coping repertoiries. Multiple regression analyses will be performed to determine if, as hypothesized, personal factors mediate dysfunctional coping behaviors; we will also examine the bi-directionality of the variables. The hypotheses which we will test in this study are:

1) Certain adaptive and maladaptive copying responses occur in response to family stress, (including structure, process and perceived stressfulness), as mediated by personal structure and status factors.
2) Certain adaptive and maladaptive coping responses occur in response to workplace stress, as mediated by personal structure and status factors.

Neuropsychological Effects of Chronic Solvent Exposure

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Psychological Disorders
5 K01 OH00028-03
09/27/85 - 09/26/88
$32,400 ($97,200 Cum)

Objectives

Discrimination of the specific neuropsychological deficits occurring with chronic low-level exposure to different neurotoxic substances and development of a conceptual framework to explicate the mechanisms of these effects are of primary importance in the field of behavioral neurotoxicology. These issues are particularly germane to individual health and welfare as knowledge about them will allow development of screening techniques and preventive health measures aimed at minimizing the neurological, behavioral, social, and safety hazards which may accompany neurotoxic exposures.

This present study will explore these areas by longitudinally examining a group of workers experiencing chronic exposure to mixed solvents in the silk screening industry. It is predicted that exposed workers will show significant deficits on neuropsychological testing in the functional areas of visuopatial reasoning and short-term memory and that they will have affective complaints. In addition, solvent exposed workers are predicted to have difficulties on tests of cognitive flexibility which are associated with frontal lobe functioning.

Methodology

This three-year study will test a group of workers exposed to a little-investigated group of mixed solvents to be compared with non-exposed workers. Following medical, neurological, and demographic screening, each worker will undergo a short (60-75 minute) battery of neuropsychological tests, including a standard screening battery and additional tests of cognitive flexibility. Retesting will be completed at 12-month intervals.

Research design is modeled after that used in a study on lead-exposed workers in order to maximize comparability of data collected on solvents to existing data on lead.

The present investigation will utilize traditional neuropsychological methodology to examine the specific cognitive deficits which may accompany chronic exposure to this group of mixed solvents, assess relationship of duration of exposure to degree and nature of neuropsychological deficits, examine changes in test performance within subjects over time, and explore the nature of cognitive flexibility in workers with this exposure. Comparisons will be made between the neuropsychological data on this population and those derived from the parallel completed study of lead workers in order to discriminate specific cognitive and affective characteristics of lead and solvent encephalopathies.

Progress and Accomplishments

To date the subject groups to be evaluated for solvent exposure have been identified and agreement to complete the study has been made with management. Preparations have been made to begin testing in 3/86. The data on a lead comparison group have been collected and analyzed.

Significance

Explication of the specific neuropsychologic impairments occurring in workers chronically exposed to a particular group of mixed solvents and comparisons of solvent and lead induced neuropsychologic dysfunction may provide clues to explicating the mechanisms (psychologic, neuropathologic, metabolic) underlying specific neurotoxic encephalopathies. In addition, tasks assessing cognitive flexibility will help determine whether cognitive rigidity may be contributing to the functional intellectual deficits accompanying exposure to these solvents and to gain further information about brain-behavior relationships by examining effects of this exposure on tasks known to be sensitive to cerebral frontal lobe dysfunction.

A Model for Predicting Glove Polymer Permeation

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Engineering Control Systems
5 R01 OH01932-03
12/01/83 - 11/30/86
$72,733 ($199,089 Cum)

Objectives

The objective of this work is to utilize physical-chemical parameters for solvent polymer systems in models to predict pertinent permeation parameters; most notably the three-dimensional solubility parameter (3-DSP) of the solvent and polymer and the molecular size and shape of the solvent. Using a multiple regression model, these parameters will be regressed against permeation parameters such as steady-state permeation rate and breakthrough time for known polymer-solvent combinations. Then, from the model permeation parameters can be predicted for unknown combinations.

Methodology

Solubility parameters for polymers are determined using a method developed by Hansen in which polymer samples are submerged in approximately 55 different solvents. Those solvents which affect the polymer most will have solubility parameters similar to the polymer. For molecular size and shape of the permeant molecule, it is realized that some combination of the two concepts will have to be used. All permeation tests have been completed using ASTM D-739 test methods. Both infrared and gas chromatographic detection techniques have been utilized.

Progress and Accomplishments

Progress has resulted in the construction of models for Viton®, butyl, and nitrile rubbers. The differences between the solubility parameters for Viton and 19 solvents were regressed against the steady-state permeation rate and breakthrough times for those solvents. In the steady-state permeation rate model, the coefficient of determination (γ^2) was 0.48 while the breakthrough time coefficient of determination was 0.42. The results were used to predict breakthrough times and permeation rates for unknown solvents. The upper and lower 95% confidence limits were calculated for permeation rate and breakthrough time respectively.

In order to attempt to improve these correlation coefficients and provide models for butyl and nitrile rubber, several variables were added to the model, including the thickness of the polymer specimen and the molecular volume of the solvent. Molecular volume was calculated using a computer model which calculated the actual space occupied by the molecule. Results of those studies were as follows:

1. Thickness was not a significant variable in breakthrough time studies.
2. Thickness was a significant variable in steady-state permeation rate studies.
3. Molecular volume was only significant for steady-state permeation rate in the Nitrile study ($P = 0.003$).
4. Molecular volume was a significant variable in all three break through time studies.
5. The difference in solubility parameters (Δ 3DSP) was always the most significant variable by a considerable margin.
6. In general, thickness and molecular volume, through significant, did little to improve overall correlation coefficients.
7. Coefficients of determination (R^2 values) for breakthrough time studies ranged from 0.38 to 0.55.
8. Coefficients of determination for steady-state permeation rate studies ranged from 0.57 to 0.64.

These results indicate that there is a significant portion of the variability in the model not explained by the variables which were studied. It is thought that much of that variability is owed to inherent "within" variation in polymer samples. Consequently, it is not likely that quantitative prediction of steady-state permeation rate and breakthrough time is possible. In particular, this is true for breakthrough time which is dependent on extraneous and unrelated variables. It is, however, recommended that three-dimensional solubility parameters be used for qualitative prediction of best polymers. This will allow selection of the best one or two polymers for a given chemical and subsequent testing of those chemicals will provide quantitative results.

Other experiments from this grant concern themselves with temperature, thickness, and mixture effects on permeation. Also, breakthrough time as an inadequate indicator of a polymer's utility was studied. In all cases, results were preliminary and are reported in the references which follow.

Significance

The results indicate that the significance of the three-dimensional solubility parameter lies in its ability to qualitatively predict the best polymer for protection against a particular chemical. However, preliminary work has shown that prediction of permeation of mixtures is much more difficult, and may not be possible with the use of solubility parameters. Molecular size and thickness played little role in the overall predictability of the model. Consequently, it is recommended that three-dimensional solubility parameters be used in the preliminary selection of all protective clothing polymers.

Publications

Perkins, JL, and Tippit, AD: Use of Three-Dimensional Solubility Parameter to Predict Glove Permeation. AIHAJ 46: 455, 1985

Perkins JL: Use of the Three Dimensional Solubility Parameter to Predict Glove Permeation. Am Ind Hyg J 46:455, 1985

Perkins, JL, Ridge, MC: Use of Infrared Spectroscopy in Permeation Tests. Performance of Protective Clothing, R.L. Barker and G.C. Coletta, eds. American Society for Testing and Materials, Philadelphia, STP-900, 1986

Perkins JL, Holcombe AB, Ridge MC, Wang WK, Nonidez, WE: Skin Protection, Viton, and Solubility Parameters. AIHAJ 47:803, 1986

Perkins JL, Ridge MC: Use of Infrared Spectroscopy in Permeation Test, in Performance of Protective Clothing, RL Barker, GC Coletta (eds), American Society for Testing and Materials, STP 900, 1986

Perkins JL, Holcomb AB, Ridge MC, Wang WK: Skin Protection, Viton, and Solubility Parameters, Am. Ind Hyg J, December, 1986

Perkins JL: Chemical Protective Clothing - Selection, Use, and Program Considerations, submitted to Applied Industrial Hygiene J, November, 1986

Fundamental Investigation of Exhaust Hoods

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Engineering Control Systems
5 R01 OH02132-03
05/01/85 - 04/30/88
$90,675 ($271,274 Cum)

Objectives

The long-term objective of this grant is the optimization of exhaust hoods used in industrial ventilation, maximizing the capture ability and minimizing the air flow through the hood. Such an approach will reduce worker exposure as well as the cost of operating the exhaust system.

Methodology

The theoretical portion of the study utilizes superimposition and conformal mapping techniques to solve the flow field equations for oblong, circular and triangular hood openings. The experimental verification of the theoretical results is carried out by the measurement of airflow using a hot wire anemometer. The flow measurements are carried out in front of wire anemometer. The flow measurements are carried out in front of specifically designed hoods to represent a wide variety of hoods. The ultimate test of the hoods will be carried out using a tracer gas.

Progress and Accomplishments

To date we have carried out the theoretical investigations leading to closed form solutions for single or multiple hood openings with flanges and with or without flanking planes. Experimental verification of the theoretical equations for hoods with or without flanking planes is completed. The experimental research on the verification of hood efficiency factors and the theoretical and experimental delineation of design parameters for the efficient design of hoods have started. The results are reported in a number of papers which are in various stages of publication.

Significance

In the results obtained to date, we were able to show that with minimal effort it is possible to reduce required flow rate for a hood by about 15 percent while increasing the hood capture efficiency to 100 percent under non-ideal conditions (cross drafts) the capture efficiency of the hood can be kept at very near to 100 percent with minimal penalty in flow rate. The complete research results should enable designers to apply the principles developed in increasing the economic and capture efficiencies of hoods.

Publications

Esmen NA: Numerical Expressions for Ventilation Parameters Ventilation 85: Proc. 1st Int'l Sym on Ventilation for Contaminant Control, HE Goodfellow (Edit) Elsevier Amsterdam p. 623-632, 1986

Esmen NA, Weyel DA: Aerodynamics of Multiple Orifice Hoods Ventilation 85: Proc. 1st Int'l Sym on Ventilation for Contaminant Control, He Goodfellow (Edit) Elsevier Amsterdam p. 735-742, 1986

Durr DE, Esmen NA, Stanley C, Weyel DA: Pressure Drop in Elbows. Applied Ind Hyg Vol. 2, No. 2 p. 57-60, 1987

Durr DE, Esmen NA, Stanley C, Weyel DA: Pressure Drop in Flexible Ducts. Applied Ind Hyg Vol. 2, No 3, p. 99-102, 1987

Esmen NA, Weyel DA: Aerodynamics of Airflow in Front of Arbitrarily Shaped Symmetric Hoods II: Experimental Study. Annual of Occup Hyg, in press

Esmen NA, Weyel DA: Aerodynamics of Airflow in Front of Arbitrarily Shaped Symmetric Hoods II: Theoretical Study. Annual of Occup Hyg, in press

Ventilation for Work in Confined Spaces

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Engineering Control Systems
1 R01 OH02329-01
01/01/87 - 12/31/89
$102,904 ($102,904 Cum)

Objectives

The overall objective of this three-year project is to study ventilation relative to work in confined spaces (CS) and to provide data and guidelines which will be useful for designing effective ventilation. The specific research objectives are as follows:

1. To conduct experimental studies involving design parameters affecting CS ventilation. These are summarized as follows:
    a. Space (CS Model) Configurations
        Open-top and closed-top
        Shape variations -- cubical and non-cubical
    b. Ventilation Schemes
        Modes -- exhaust and supply
        Inlet/outlet elevations -- high, central, low
        Volume flowrates -- "air changes" per hour
    c. Contaminant Characteristics
        Emission rates -- steady-state or time-dependent
        Concentrations -- low-level, oxygen deficient, heavier-than-air
2. To characterize ventilation effectiveness by determination of experimental/empirical air mixing factors (K-factors) for calculations of dilution ventilation design.
3. To develop a user-friendly computer design model for CS ventilation.
4. To develop and evaluate a contaminant dispersion model for CS ventilation.
5. To conduct field studies of CS ventilation in Year 3 of the project for evaluation and refinement of the computer design model.

Methodology

Laboratory studies are being conducted using models of basic CS configurations. The models permit variation of CS design parameters (open-top vs. closed-top, cubical and non-cubical shapes) and ventilation schemes (exhaust vs. supply, inlet/outlet elevation, volume flowrate). Dilution ventilation effectiveness is determined for different simulated atmospheric conditions inside the CS model (low-level "toxic", oxygen deficiency, heavier-than-air. Measurements of contaminant concentrations, contaminant release rates (steady-state, time-dependent), and ventilation rates permit calculation of the air mixing factor (K-factor), which is anticipated to be a function of the CS configuration, ventilation scheme, and contaminant characteristics. The experimental studies will yield a database of K-factors pertaining to CS ventilation design.

Simulation and analysis of "contaminated" CS atmospheres will be carried out by several means: 1) low-level "toxic" concentrations using SF with analysis by a gas chromatograph (GC) with an electron capture detector (ECD), 2) oxygen (O_2) deficiency will be accomplished by elevated nitrogen (N_2) levels, with analysis by a 4-channel electrochemical O_2 sensor system, and 3) the methodology for heavier-than-air characteristics will be determined pending other findings; analysis will be either by GC or a multi-channel sensor system.

A computer model will be developed as an aid for designing CS ventilation. It will strive to be user-friendly and will incorporate the experimental/empirical database of K-factors and qualitative guidelines from laboratory and field studies. Another computer model will be developed to represent contaminant dispersion inside a CS. This model will utilize results of experimental airflow studies, Finite Element Method (FEM) airflow approximations, and contaminant diffusion characteristics.

Predictions from this model will be evaluated against experimental studies of contaminant concentration inside a ventilated CS model.

Progress and Accomplishments

Activities in Year 1 of the project were largely devoted to design and construction of experimental facilities, preliminary testing, and development of preliminary computer models. Principal facilities included CS models, model ventilation systems, analytical systems 9GC-ECD and O_2 sensor), a multiple-point air sampling system, and equipment for airflow pattern ("smoke") testing and velocity measurements. Preliminary testing of the GC-ECD involved several major difficulties, with corrections required to be made by the manufacturer. These difficulties confirmed a need to develop another (non-GC) method for experimental analysis, hence, the development of a 4-channel O_2 sensor system.

Studies of airflow characteristics for cubical CS models yielded both anticipated effects and effects which were difficult to anticipate and describe in a precise manner. Significant differences were observed between exhaust and supply ventilation, and for different inlet/outlet elevations inside a CS model.

Computer model development included a preliminary program for CS ventilation design and a FEM program for airflow velocity data inside a cubical CS. Substantial further development is planned for the ventilation design model and for a contaminant dispersion model.

Several important results are anticipated from this study, but have not yet been accomplished; these include:

- A useful and expandable database of air mixing (K) factors for dilution ventilation design will be established as a function of CS configuration, ventilation scheme, and contaminant characteristics.
- Design guidelines for CS ventilation will be obtained from laboratory and field studies.
- A user-friendly computer design model for CS ventilation will utilize the K-factor database and guidelines, and will be evaluated against field studies.
- Feasibility for development of a contaminant dispersion model will be evaluated and applied specifically for a CS.

Significance

The results of this study will be significant in two primary ways:

- Better design methods will help to bring about more effective ventilation for work in CS. Air contaminants are the leading cause of deaths and injuries during work conducted in confined spaces. Ventilation is the primary engineering control action to reduce airborne hazards, and very little is presently known about ventilation design specifically for CS.
- The primary accomplishments anticipated from this study (highlighted above) can be extended to non-CS situations and represent a foundation for subsequent studies which could be conducted for other work places.

The Impact of Separation on Exposure and Hood Capture

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Engineering Control Systems
1 R01 OH02392-01
09/29/87 - 09/28/89
$57,245 ($57,245 Cum)

Summary

The research described in this proposal is designed to develop a mathematical model that will enable the industrial hygiene engineer to make reliable estimates of personal exposure to individuals who are working at operations under the control of local exhaust hoods. The proposal focuses on the importance of the worker as an obstruction in the flow field of the hood, and how the phenomenon of boundary layer separation influences exposure. The long-range objective is the development of optimal hood designs through an understanding of the factors that impact on the hood's ability to control exposure. The term optimal refers to achieving a desired level of control at the lowest cost. Specific aims include the development of personal exposure models for uniform flows such as would be found in spray booths, and also exposure vs. capture efficiency models for flanged circular hoods. The effect of crossdrafts and the position of the worker on exposure will also be examined.

Capture Efficiency of Local Exhaust Ventilation Systems

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Engineering Control Systems
5 R03 OH02101-02
04/01/85 - 03/31/87
$14,175 ($32,545 Cum)

Objectives

1. Development of a Theoretical Model of the three dimensional velocity field into a flanged circular hood, both with and without a crossdraft blowing perpendicular to the hood centerline.
2. Empirical validation of the above model.
3. Development of a theoretical model of capture efficiency based on the validated flowfield model using computer aided design.
4. Validation of the capture efficiency model.

Methodology

A modified potential flow solution was developed for the flow field into the flanged circular hood. An empirical validation was conducted using hot film anemometry for hoods operating with and without crossdraft. BASIC programs have been written for the IBM XT and AT personal computers that enable graphic plots of the flowfield to be displayed in two dimensions. These plots were used to construct models of capture efficiency, which were evaluated using a Sulfur Hexafluoride tracer gas system with portable Gas Chromatograph.

Progress and Accomplishments

All objectives are complete except a field validation of the model.

Significance

The work presents a method for assessing the effects of crossdrafts on hood performance and quantitatively evaluating a hood's performance. The potential for determining the adjustments needed to a given system in order the reduce the employee's exposure from one level to another are discussed.

Publications

Flynn MR, Ellenbecker MJ: The Potential Flow Solution for Air Flow into a Flanged Circular Hood. Am Ind Hyg Assoc J 46(6):318-322, 1985

Flynn MR, and Ellebecker MJ: Capture Efficiency of Flanged Circular Local Exhaust Hoods. Ann Occup Hyg 30 (4):497-513, 1986

Flynn MR, and Ellenbecker MJ: Empirical Validation of Theoretical Velocity Fields into Flanged Circular Hoods. Am Ind Hyg Assoc J 48(4):380-389, 1987

Tractor Stability Information Processing System

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Engineering Control Systems
1 R03 OH02236-01A1
09/29/87 - 09/28/89
$20,778 ($20,778 Cum)

Summary

The long-term objective of this research is to reduce fatal farm tractor overturn accidents. The first step toward the long-range goal has been completed. A new concept hypothesized from the initial model tractor testing is that a roll roughness coefficient, C, can be used to predict ground irregularities before an irregularity is struck by a tractor wheel. This project represents the second step toward the long-term goal and has three parts. One is to build a fully enhanced model tractor to verify and refine equations that continually provide tractor operators with stability and instability information. The second part is to develop display interface as to the timing, weighing, and nature of instability signals. The third part will be the verification and definition of the roll roughness coefficient on full scale tractors on varying terrains. The results of this project will serve as the basis for the third and final step: the development of a fully operative information processing system which will aid operators in maintaining vehicle stability.

Reducing Solvent Exposure of Auto Body Workers

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Engineering Control Systems
1 R03 OH02422-01
09/29/87 - 09/28/88
$21,887 ($21,887 Cum)

Summary

Auto body paint shops represent a large industry whose employees regularly face numerous potential health hazards including significant exposure to volatile organic solvents used in auto body spray painting activities. Solvent exposure may cause acute and chronic adverse neurological effects. Respirators are frequently used in auto body paint shops; gloves are seldom worn. In order to lower employee exposures to volatile organic solvents it will be necessary to demonstrate to shop owners and employees that respirators and gloves can be used effectively to reduce solvent exposures in auto body spray painting operations. A study will be conducted with the participation of 12 to 16 auto body spray painters and assistant painters in 4 to 6 auto body repair shops. High exposure activities for which gloves and respirators are appropriate will be identified through observation, air sampling, and discussion with employees and manager. Employees' breath will be sampled for exhaled organic solvents. An intervention program of increased glove and respirator use will be instituted. Breath samples will again be taken. Breath sampling will serve as both a motivator for glove and respirator use, and as a means of evaluating the effectiveness of such use.

Noise Control for Honing and Other Machines - Phase II

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Engineering Control Systems
5 R44 OH01951-03
09/15/85 - 08/31/87
$86,514 ($221,314 Cum)

Objectives

The major long-term objective of this program is to provide source noise control for machine tools such that operators will be protected against permanent hearing loss due to noise exposure. The primary aims of this research are:

1. Develop a noise control treatment for the honing machine,
2. Identify machines with similar noise generating mechanisms, and
3. Develop specific noise control treatments for the selected machines.

Methodology

In our Phase I study, we determined the method by which tonal sounds are generated by honing machines. We also identified a number of potential noise control treatments including: Lanchester dampers, dynamic absorbers, increased structural damping, and increased structural stiffness.

In Phase II, which began September 15, 1985, these noise control treatments are being developed with careful consultation with machine design engineers, damping consultants, and machinery operations specialists. A number of different concepts will be analyzed and tested both in our laboratory and in the field. Only the most successful will be marketed in Phase III.

In addition to the honing machine, we will develop noise control treatments for a number of other machines with the same type of sound generating mechanisms.

Progress and Accomplishments

During the first 15 days of this project (15-30 September 1985), we presented the results of our Phase I work at Inter-Noise 85.

Significance

There are about 18,000 operators exposed to honing noise; this noise exposure can be reduced as a result of the research in Phase II. If the analysis techniques used in analyzing the honing machines are applied to other machine tools, additional employees will benefit from noise control -- about 500,000 employees in total.

New Methods for Quantitative Respirator Fit Testing

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Respirator Research
2 R01 OH01301-04A1
9/29/82 - 8/31/90
$183,006 ($388,907 Cum)

Summary

The principal aim of this grant renewal is to answer several basic questions regarding quantitative respirator fit testing, to subsequently make recommendations for optimal test procedures with special focus on particle size, probing method and compact, low-cost instrumental techniques and, finally, to implement these recommendations. The principal questions to be answered are concerned with the particle-size dependence and magnitude of aerosol leakage into the respirator cavity during inhalation, the aerosol concentration of the exhalation flow, the complex and incomplete mixing of the flows in the respirator cavity, and the relationship of probed concentration measurement to these flows.

In the renewed research, the respirator fit testing facility will be upgraded for exposure to aerosols of different type and particle size. The particle size measurement system will be upgraded and will consist of an aerodynamic particle sizer, a laser spectrometer and a differential mobility analysis system, all computer-interfaced and covering a particle-size range of 0.02 μm to about 10 μm. Coupled into this facility will be portions of the aerosol deposition study facility so that the respiratory tract exhalation flow into the respirator cavity can be determined experimentally.

The long-term objective of this research is to develop a quantitative respirator fit testing procedure which is reliable and measures accurately the protection factor the worker will have in the actual environment.

Publications

Tackett DL: Quantitative Respirator Fit Testing Using Ultra-fine Particles, M.S. Thesis, University of Cincinnati, 1984

Holton PM: Particle Size-Dependent Leakage Through the Faceseal of Negative Pressure Half-Mask Respirators, Ph.D. Thesis, University of Cincinnati, 1986

Holton PM, Tackett DL, Willeke K: Particle Size-Dependent Leakage and Losses of Aerosols in Respirators, Am Indus Hyg Assoc J in press Oct 1987

Holton PM, Willeke K: Respirator Fit Factors from Different Aerosol Size Distributions and Measurement Methods, Am Indus Hyg Assoc J in press Oct 1987

A Fundamental Study of Respirator Air Filtration

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Respirator Research
5 R01 OH01485-03
09/23/83 - 12/31/87
$98,842 ($269,897 Cum)

Objectives

The principal aim of this research is to study the characteristics of particulate air filters of the type used in respiratory protection devices. Commercially available respirator air filters will be studied to determine their efficiency as a function of particle size in the size range of interest in respiratory protection, namely 0.01 to 10 μm. In addition, the resistance of the filter media and their dust holding capacities will also be measured, and the role of electrical charge in the conventional mechanical and electrostatic filters will also be determined. A further aim of this research is to make a comparative study of several filter testing methods based on modern aerosol instrumentation and to develop one or more of these methods to the point where they can be routinely used for filter evaluation.

Methodology

Two approaches have been used to measure the filter efficiency as a function of particle size. In one approach, a monodisperse aerosol is generated by means of a vibrating-orifice aerosol generator or an electrostatic classifier aerosol generator. The aerosol concentration upstream and downstream of the filter is then measured with an optical particle counter or condensation nucleus counter. In a second approach, a polydisperse aerosol is generated by an atomizer and the concentration and size distribution of the aerosol upstream and downstream of the filter measured with a single particle optical counter. From this, the efficiency vs. particle size curve is determined. The flow rate through the filter is also varied to study the effect of flow rate on filter efficiency.

The filter loading study is made by generating a concentrated aerosol by atomization and loading the filter with this concentrated aerosol. Upon reaching a certain loading level, the filter with the loaded dust particles is analyzed to determine their efficiency as a function of particle size. In addition, the filter pressure drop is also measured. From this, the effect of dust loading on filter pressure drop and efficiency is determined.

The theoretical study consists of solving the Navier-Stokes equations on the computer to determine the filter pressure drop as a function several filters parameters. The filter efficiency and the influence of electrical charge on filter efficiency will also be determined numerically. The result will then be compared with the experimental data generated in this program.

Progress and Accomplishments

An experimental system for respiratory filter performance evaluation has been successfully developed during the first year and the system has been applied in the second year to the study of the efficiency and pressure drop characteristics of several commercially available respirator filter. The data are being analyzed for comparison with filtration theory. In addition, filter pressure drop calculation was initiated during the second year for the case of rectangular fibers found in some commercial respirators using permanently charged filter fibers.

During the third year, the focus is on completing the measurement of filter efficiency and pressure drop, and the development of the filter pressure drop and efficiency models. Comparison of the theoretical and experimental results will also be undertaken during the third year. The result of the research will also be documented for publication in journals.

Significance

The end result of this research will be an improved understanding of the characteristics of respirator air filters in general and the availability of improved filter testing methods with potential industrial and practical applications. The research should benefit further respiratory protection device development and encourage the correct use of these devices for health protection in the work place.

Publications

BYH Liu: Performance of High Efficiency Respirator Air Filters. Proc International Symposium-Workshop on Particulate and Multi-Phase Processes and 16th Annual Meeting of the Fine Particle Society. p 45-1, Miami Beach, April 22-26, 1985.

Respirator Performance Model for Particulates

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Respirator Research
5 R01 OH01595-03
09/23/83 - 02/28/87
$62,011 ($164,867 Cum)

Objectives

The overall objective of this grant is to provide a better understanding of the effect of particle size on the performance of air-purifying respirators for protection against particulate exposures. This is accomplished through experimental measurement of filter and leak performance as a function of particle size and flow rate and the use of these data in a computer model to predict overall performance for a respirator with a given size and type of leak as a function of airborne particle size distribution and work rate of the wearer.

Methodology

The first phase involves measurement, in the laboratory, of the performance of respirator filters and facial seal leaks as a function of particle size using an oleic acid test aerosol and a high resolution optical particle counter. Filter measurements are made at 7 steady flow rates from 2 to 150 L/min and 14 particle sizes from 0.1 to 11.0 μm. Leakage measurements are made at these conditions for three types of leaks and four leakage rates.

The second phase uses these data to develop a computer model that gives total penetration (filter penetration plus leakage) as a function of particle size for any inhalation flow rate, leak type and size. The model is integrated over the breathing cycle associated with five work rates and over log normal size distributions commonly found in industry. The result gives theoretical protection factors for any particle size distribution and for specific jobs where data on work rate, particle size distribution, and leakage rates are available. The model will be applied in conjunction with existing respiratory deposition models to give protection factors based on total and regional deposition as a function of work rate and particle size distribution.

Progress and Accomplishments

The experimental phase of this project has been completed. The model development phase is now complete. The model consists of programs, spread sheets, and regression equations that allow one to predict overall respirator performance as a function of work rate and aerosol size distribution based on QNFT measurement. We have evaluated applications of the model to various industrial situations and to the distribution of fit factors found in industry. Four peer-reviewed papers arising from this project have been published.

Significance

The ability of airborne particles to penetrate the filters and facial seal leaks of air-purifying respirators depends strongly on particle size as does the respiratory hazard associated with these particles. Thus, the actual protection factor obtained for airborne particles will depend on the particle sizes present in the work place environment. The respiratory hazard depends on the efficiency of deposition at various sites in the lung and is affected by both the reduction in concentration and the change in size distribution of inhaled aerosol caused by the respirator. With a few exceptions current respiratory protection factors do not distinguish between exposures to aerosols of different particle size. This study characterizes the effect of particle size distribution on the performance of representative single-use and half mask respirators. No general data exist to describe the performance of respirators as a function of particle size or particle size distribution.

Publications

Hinds WC, Kraske G: Performance of PMS Model LAS-X Optical Particle Counter (extended abstract) in Aerosols: Science, Technology and Industrial Application of Airborne Particles. Liu, Pui, Fissan, Eds, p 31-34, Elsevier, New York. Presented at First International Aerosol Conference Minneapolis, MN, Sept. 17-21, 1984

Hinds WC, Kraske G: Evaluation of Nebulizers for Use With Optical Particle Counter. Presented at the 1985 meeting of the American Association for Aerosol Research in Albuquerque, NM, November 18-22, 1985

Hinds WC, Kraske G: A Bench-Scale Aerosol Test Chamber. Appl. Ind Hyg Vol 2 p 13-17, 1987

Hinds WC, Kraske G: Performance of Dust Respirators With Facial-Seal Leaks: I. Experimental, Am Ind Hyg Assoc J 48, 836-341, 1987

Hinds WC, Bellin P: Performance of Dust Respirators With Facial-Seal Leaks: II. Predictive model, Am Ind Hyg Assoc J 48, 842-847, 1987

In-Mask Monitors and Respirator Breakthrough

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Respirator Research
5 R01 OH01603-03
09/23/83 - 11/30/86
$131,948 ($405,539 Cum)

Objectives

The objective of this program is to demonstrate that small, chemically sensitive colorimetric films being developed by Moleculon can be combined with miniature electro-optic sensing technology to provide a compact, convenient and reliable method for monitoring in-mask levels of hazardous gases and vapors in air purifying respirators. Such monitoring can both alert the user to early signs of respirator failure, and provide increased safety for the use of cartridge filters close to, but not beyond, their end of life.

Methodology

Initial research has been devoted to better quantifying the response of formaldehyde-sensitive films, with much emphasis on the thin-film chemistry involved, and the sensitivity and stability obtained. Further work has examined the applicability of the same concept to another hazardous gas, hydrogen sulfide. Currently under investigation is the actual use of the formaldehyde sensing system to detect and monitor end of cartridge life for formaldehyde removing cartridges, with emphasis on the experimental test apparatus involved and accurate supporting measurements of low formaldehyde concentrations.

The chemistry underlying the formaldehyde sensing system is a highly modified, nonaqueous Shiff reaction scheme free from hazardous components. The hydrogen sulfide sensor relies on the reaction of certain bismuth salts with hydrogen sulfide in organic solution, bismuth being unusually nontoxic among the metal ions colorimetrically sensitive to sulfide vapor. Both the sulfide and formaldehyde systems are continuously monitored by a tiny dual-beam spectrometer and its associated electronics.

Progress and Accomplishments

Both the formaldehyde and sulfide systems appear to have the sensitivity required for adequate in-mask and cartridge end of life use. The stability and sensitivity of the formaldehyde sensing film have shown fluctuations requiring extensive chemical and coating research. Tests now under way with real respirators are expected to demonstrate the merit and high utility of this approach.

Significance

There are many industrial settings where the use of respirators is the only immediate option for respiratory protection, yet too little is known about the service life of air purifying cartridges toward particular challenges. This leads to uncertainty in the level of protection provided by current masks, requires the uneconomical disposal of cartridges which may retain more than adequate protective capacity, and limits the certification of protective masks for broader use. The kind of inexpensive, in-mask monitoring under investigation here is intended to address all of these important concerns, and will in time greatly increase our understanding of the factors determining cartridge reliability.

Measure of Work Performance Decrement Due to Respirators

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Respiratory Research
1 R01 OH01632-01A2
03/01/87 - 04/30/89
$134,247 ($134,247 Cum)

Summary

Measurement techniques will be tested to establish an evaluation method which can quantitatively assess the decrement in work performance resulting from the wearing of respirators. Such an assessment technique would be useful for evaluating the effectiveness of the respirator in terms of worker acceptance as well as worker productivity, two key goals of any industry's respiratory protection program. A homogeneous group of young, healthy, non-smoking males will be monitored while they are wearing four categories of respirator states and performing three levels of simulated work tasks. The respirator and task conditions are selected to represent situations typical of industrial applications. In addition to determining the work performance decrement for each task level with each respirator, two other types of data, physiological and subjective evaluations, will be collected to test for any correlation between them and work performance. A quantitative measurement tool to determine the extent of work performance decrement due to a respirator would add important respirator usage evaluation information to the present methods of respirator evaluation. Development of such a technique has long-term potential for studying other critical areas of respirator use such as emergency and firefighting, for input in respirator design and for evaluation of worker acceptance.

Optimal Design of Respirator Cartridges

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Respirator Research
5 R01 OH01644-03
04/01/84 - 03/31/87
$76,656 ($211,038 Cum)

Objectives

Respirator cartridge performance is examined through the fundamental theories of mass transport, as used by chemical engineers and physical chemists, to determine optimal design parameters for respirator cartridges.

Methodology

An experimental system consisting of a vapor generator, dilution air stream, charcoal beds, and vapor detector allows the performance of respirator cartridges to be monitored continuously. Mathematical modeling of the observed performance of respirator cartridges as a function of time permits the geometry of the respirator cartridges to be systematically modified to give optimum performance.

Progress and Accomplishments

We developed, using a microcomputer, a rapid method of determining the basic factors, (i.e., the adsorption coefficient and mass transfer efficiency) which control the performance of a respirator cartridge. This computer program was then used in analyzing the performance of respirator cartridges exposed for extended periods of time to high concentrations of contaminant, in a rapidly flowing stream of air, at humidities as high as 100%.

In the process of analyzing these data, we developed the first satisfactory theory for the performance of a respirator cartridge at extremely high relative humidities. This equation is as follows: $W = Wo \exp(KE^2)$, where W = gm uptake/gm adsorbent, p = density of liquid solvent, and E = potential energy for adsorption. The potential energy for adsorption from a humid atmosphere is $E/RT = (1+K_1)\ln(P_s/P) + K_2 \ln(RH/100)$, where P is the vapor pressure of the solvent, P_s is the vapor pressure 63 of the liquid solvent, T is the absolute temperature, R is the ideal gas constant, and RH is the relative humidity, and K_1 and K_2 are constants describing the effect of the ambient humidity on the adsorption of the vapors of the solvent. By the above equation, the adsorption coefficient can now be determined at any temperature, vapor concentration, and relative humidity. A very important result of the above theory is that when the RH = 100%, the term containing K_2 vanishes; the resultant equation under these conditions gives a simple correlation between the adsorption coefficient from dry air and from air totally saturated with water vapor. This equation fits experimental data for the adsorption of trichloroethylene at relative humidities ranging from 5 to 85% and concentrations ranging from 300 to 1300 ppm, to within 2% error.

An important prediction from the above equation is that even in an atmosphere saturated with water, a respirator cartridge may have a useful residual efficiency. This was proved experimentally using vapors of xylene, methy ketone, and ethylacetate. For all three vapors, protection for 8 hours could be shown using a standard size respirator cartridge in an air flow of 20 lpm of air completely saturated with water vapor. The key factor learned from this work is that instead of writing off the use of respirator cartridges at high relative humidities, we should determine the circumstances under which they will be effective. Protection against benzene under the same conditions was studied in detail with the standard respirator cartridge, because of the very poor mass transfer of benzene vapors (number of theoretical plates remaining below ten), protection for 8 hours could not be demonstrated. However useful protection for 4 hours could readily be obtained. Next it was found that a change in the geometry of the respirator cartridge permits a respirator cartridge containing 40 grams of adsorbent to protect against benzene in air at 100% for more than 8 hours. This demonstrates the importance of understanding the kinetic factors influencing mass transfer.

Significance

By modeling and designing respirator cartridges to operate under adverse conditions, we increase our confidence in their safe use in more normal circumstances. Particularly important is the equation giving the adverse effects of a high relative humidity. As the experiments with benzene showed, ignoring relative humidity may cause unacceptably high exposure to occur without any warning.

Publications

Underhill DW: Pulse Residence in Short Chromatographic Columns. Anal Chem 57:826-9, 1985

Underhill DW, Forbes SL: Modeling Adsorption Bed Behavior Using a Microcomputer. J Air Pollut Control Assn 36:61-4, 1986

Underhill, DW: Calculation of the Performance of Activated Carbon at High Relative Humidities. Am Ind Hyg Assoc J 48: 909-913, 1987

A Model and Tests for Prediction of Respirator Absorbent Behavior

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Respirator Research
5 R01 OH01646-03
09/23/83 - 08/31/87
$119,866 ($329,300 Cum)

Objectives

The Wheeler-Dubinin adsorption model has been proposed for use in the workplace to predict breakthrough times of activated carbon-containing respirator cartridges. The Wheeler-Dubinin Model has been shown to be an accurate predictor of adsorptive behavior (within ± 15%) for small carbon masses (<10 g) with a single adsorbable vapor, low volumetric flow rates (<0.05 L/s) and high inlet concentrations (>10,000 ppm). The purpose of these investigations is to extend the Wheeler-Dubinin Model to realistic conditions of use, i.e. multiple adsorbable vapors (binary vapor mixtures with water as the second vapor), large adsorbent masses (30-40 g), high flow rates (1-3.3 L/s), etc., so that accurate predictions of respirator adsorbent cartridge behavior can be made.

Methodology

Carbon adsorbents are first characterized using a reference vapor such as carbon tetrachloride. Once the reference adsorbent has been characterized, it can be used in the application of the Wheeler Kinetic breakthrough model. The kinetic data are developed by passing vapor laden-air through an adsorbent bed. The effluent is monitored until a chosen exit-to-inlet ratio (<0.04) is achieved. These data are then plotted as the natural log of the exit-to-inlet ratio versus the time. From this curve, the rate constant and the kinetic capacity are determined. These data are then used to predict the behavior of other vapors. The effect of water vapor will be determined in two modes, one in which water vapor is pre-equilibrated with the adsorbent and a second which utilizes dry carbon. These adsorbents are then challenged with vapor-laden (a binary mixture of the test adsorbate and water vapor at the test concentration) air. Again, the effluent is monitored until a chosen exit-to-inlet ratio (<0.04) is achieved. These data are then plotted as the natural log of the exit-to-inlet ratio versus the time. From this line the rate constant and the kinetic capacity are determined. The data can then be used to predict the behavior of other vapors in the presence of water vapor.

Progress and Accomplishments

Major progress has been made in completion of all phases of the proposed work.

In Phase I, the high flow/large adsorbent mass gas/vapor system for challenging adsorbent was constructed and tested. Initial data were developed on the generation of mixtures of vapors. Preliminary data on the effects of water vapor on adsorption of the standard reference vapor, carbon tetrachloride, have been developed. These data indicate that water vapor at low concentrations (RH's below 50%, 23°C) can adversely effect the predictions of the model.

In Phase II of the investigation, the stability and reproducibility of the large bed system were established. An additional investigation was completed which focused on the reproducibility of respirator adsorbent packing-volumes and compared the extent of carbon activation. This demonstrated that commercial activated carbon-containing respirator cartridges from three of the major suppliers are associated with significant differences in the degree of adsorbent activation and in the amount (as determined by the mass of adsorbent) of carbon in the cartridge.

The influence of adsorbate polarity on reference vapor selection was investigated. Polarity may be important for reference vapor selection due to the influence of electrostatic forces in the adsorption of polar vapors. Adsorbate polarity was investigated by determining the adsorption isotherms and characteristic curves for the three isomers of dichlorobenzene. In terms of molecular weight, vapor pressure, density, and electronic polarization, the three isomers are very similar; the major difference being the range of dipole moments: 1,2-dichlorobenzene, 2.50 D; 1,3-dichlorobenzene, 1.72 D; and 1,4-dichlorobenzene, 0.00 D. If adsorbate polarity influenced adsorption, the three curves should have different slopes. The slopes of the three curves were not statistically different at the 95% level. It was concluded, therefore, that electrostatic forces do not influence vapor adsorption when adsorbate polarities are between 0 and 2.50 D, and a single reference vapor can be selected for breakthrough prediction.

In Phase III, hand packed activated carbon beds similar in size to respirator cartridges were characterized with carbon tetrachloride. The 1% breakthrough times for benzene, n-heptane, and ethyl acetate were predicted and experimentally verified using a modified Dubinin/Wheeler Model.

The Dubinin/Wheeler Model was modified to account for increased axial dispersion in large carbon beds. Increased axial dispersion resulted in a decrease in the ratio of the kinetic saturation capacity at 1% to complete breakthrough. Calculation of breakthrough time for small beds assumed this ratio was equal to 1.0. This assumption was found to be invalid for large beds. The modified Dubinin/Wheeler Model was used to predict 1% breakthrough times for activated carbon beds similar in size to organic vapor air-purifying respirator cartridges at environmental conditions consistent with respirator use. Breakthrough times were found to deviate from experimentally determined values by as much as 38%; with the average deviation equal to 16.5.

Significance

Currently, there is no reliable method for predicting the adsorptive behavior of respirator adsorbent cartridges in use. Although a number of approaches to this problem have been attempted, none have been demonstrated to be widely successful. The Wheeler-Dubinin model has been demonstrated to be an accurate predictor of adsorbent bed behavior. The continued development of this model will permit its general application in the field, thus filling this void.

Publications

Trout D, Breysse PN, Hall TA, Corn M, Risby T. Determination of Organic Vapor Cartridge Variability in Terms of Degree of Activation of the Carbon and Cartridge Packing Density. Am Ind Hyg Assoc J, 47 491-496, 1986

Breysse PH, Hall TA, Corn M, Risby T, Jonas L: Prediction of Activated Carbon Bed Service-Life Using the Dubinin/Wheeler Model. Am Ind Hyg Assoc J, 1986, submitted

Breysse, PN, Cappabianca AM, Hall T: Effect of Polarity on the Adsorption of Dichlorobenzene Isomers, 1987, in press

Hall TA, Breysse PN, Corn M, Jonas LA: The Effects of Adsorbed Water Vapor on the Subsequent Adsorption of CC1 Vapor. Am Ind Hyg Assoc J, 1985, in press

Respirator Tolerance

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Respirator Research
2 R01 OH02005-04
08/01/84 - 09/15/90
$118,246 ($352,868 Cum)

Summary

The determinants of respirator tolerance appear to be multifactorial. This study will utilize a human panel method for the assessment of the integrated effects of respirators and will examine the effects of several types of respirator loads to gain understanding of the mechanisms by which workers tolerate respirator use. Panels of human subjects will participate in a series of studies. Outcome measures will include physiologic variables (e.g., respiratory timing, ventilation, ventilatory work), subjective tolerance (measured by visual analog scales), and work performance ability. Psychophysiologic sensitivity to loads (both resistive and pressure bias) will be measured as well. Studies will be performed in a laboratory setting, in a prescribed field course (during which the subject may self pace), and at worksites. Respirator surrogate loads and actual respirators will be employed, and several types of exercise will be employed. The inter-relationships between and among the predictor (e.g., respirator load, exertion type) and outcome measures will be assessed to define mechanisms of respirator effects and to develop accurate and reasonably simple means for human panels to test respirators. The experimental design permits complex analyses by having each subject participate in a series of studies and by repeating certain experimental conditions over time.

Subprojects will address additional specific problems. The effects of pressure biasing (as occurs with many SCBA's) upon physiologic, subjective, work performance, and lung volume measures will be tested. A group of self-identified workers who are particularly intolerant of respirators will participate. The use of respirators in simulated "emergency use" situations will be assessed in comparison to more routine use.

Publications

Harber P, Tamimie J, Bhattacharya A, Barber M: Physiologic Effects of Respirator Dead Space and Resistance Loading, J of Occupational Medicine Vol. 24, No. 9 September 1982

Harber P, SooHoo K: Static Ergonomic Strength Testing In Evaluating Occupational Back Pain, J of Occupational Medicine Vol. 26, No 12 December, 1984

Asbestos Fiber Collection by NIOSH-Approved Respirators

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Respirator Research
5 R01 OH02154-02
01/01/86 - 12/31/88
$49,254 ($134,707 Cum)

Objectives

The goal of this research is to use an asbestos aerosol to investigate the performance of respirators certified for use with asbestos. Four manufacturers' certified air-purifying respirators will be tested. Since asbestos is a very hazardous material and since such respirators are widely used to protect workers from asbestos exposure, it is very important that certified respirators perform as expected. Unfortunately, the present NIOSH certification procedure does not require that respirators be tested against asbestos. It is the goal of this research to investigate the significance of this and other possible deficiencies in the certification procedure.

Methodology

The performance of the respirators against asbestos will be compared to that for silica, the standard dust used in the NIOSH certification procedure. Respirator penetration will be measured under steady flow conditions, as is done in the certification test, and under cyclic flow, to simulate actual use conditions. Three replicate tests will be performed for each combination of test aerosol and flow pattern, so that twelve tests will be performed for each respirator. The measurements will be analyzed by standard statistical techniques, including analysis of variance, to give the effects on performance of aerosol type, flow pattern, and respirator model.

Progress and Accomplishments

Exploratory experiments using polystyrene latex spheres to determine the collection efficiency of ten manufacturers' filters have been completed. From these ten manufacturers, four were chosen for the silica and asbestos tests. The tests with silica at steady flow have been completed. The asbestos penetration experiments at steady flow are in progress. It is expected that they will be completed by December, 1987. These tests will be immediately followed by tests of silica and asbestos under the cyclic flow condition. Results from the tests of penetration with latex spheres and measurements of filter characteristics are being combined to predict the collection of silica and asbestos using the single fiber efficiency model. These predictions will be compared to the results obtained from the experiments with silica and asbestos under steady flow.

Significance

The results from these experiments will be useful to those using respirators for protection from asbestos, to regulatory agencies for developing recommendations for respirators in asbestos atmospheres, to certification laboratories for developing appropriate tests to approve respiratory protection equipment, and to manufacturers for designing respirators for fibrous aerosols like asbestos.

Publications

Brosseau LM, Evans JS, Ellenbecker MJ: Poster Session, Asbestos Fiber Collection By NIOSH-Approved Respirators. American Industrial Hygiene Conference, Montreal, June 1986

Quantitative Respirator Fit Test by Negative Pressure

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Respirator Research
1 K01 OH00068-01
08/01/87 - 07/30/90
$ 32,400 ($32,400 Cum)

Objectives

The principal objective is to investigate the capabilities of a new quantitative respirator fit test method that seems to be less complex, more portable and more cost-effective than current methodologies. The new method is based on exhausting air from a temporarily sealed mask, thereby generating a negative pressure that replicates the inspiratory driving force for leakage into the mask. The exhaust flow rate that is required to generate and sustain a pre-selected negative pressure in the mask is a direct measure of the mask leakage flow rate, which can be used as an index of respirator fit.

Additional objectives include defining inspiratory pressures and flow rates for various air purifying media over a range of metabolic work rates, and determination of actual workplace respirator fit factors.

Methodology

Sensitivity and precision limits of the new method will be determined in a sequential comparison with a computerized aerosol fit test system based on corn oil. A test population recruited from Air Force personnel already assigned to a respiratory protection program will be used and anthropometrically compared to a defined respirator test panel.

Capabilities of the new method to measure respirator fit representative of non-rest work rates will be examined with a computerized open-circuit spirometric technique. Actual workplace measurements of respirator fit will also be attempted with the new method.

Progress and Accomplishments

Design, fabrication, and checkout of an automated negative pressure fit test system is on schedule and essentially complete. The comparative test with the aerosol system will begin in December 1987.

Significance

If the sensitivity and precision of the negative pressure fit test technique is validated, a straight-forward method for measuring respirator fit could be made available to a broad range of industrial respirator users. The data derived with the system could significantly improve respirator selection, fitting, training, and use.

An Advanced Respiratory Protective Device

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Respirator Research
1 R43 OH02312-01
09/30/86 - 06/15/87
$48,629 ($48,629 Cum)

Objectives

Protection of workers and other persons from exposure to airborne chemicals is becoming an increasingly important concern in industrial, medical, and indoor air environments. Frequent illness and even death can be traced to acute high level chemical exposure. In addition, chronic health problems and cancer may result from long-term, low-level exposure to certain airborne compounds and chemical mixtures.

Filters and respirators worn over the face are used as protection against toxic vapors in many occupations. At present, there is no fast and accurate way to determine the status of a used or partially used respirator device. Proposed approaches to estimating the useful life have included accurate logging of use time, periodic breakthrough testing, and color change indicators. These ideas are just estimates and, as is the case with breakthrough, can result in the exhaustion of the filter, making the respirator useless. The ideal solution to this problem has been recognized by NIOSH for a long time and consists of a filter canister which incorporates an indicator (i.e., a sensor) that signals the end of the respirator's useful lifetime.

The overall goal of this work is to build an "Advanced Respiratory Device". This is an adsorbent canister that can fit into a gas mask and contains a microsensor to warn the user prior to hazardous gas exposure. The adsorbent technology is well known and, thus, the key to this capability is the feasibility of a small, low-cost, low-power microsensor that fits inside the adsorbent bed or gas mask and has the proper analytical characteristics (detects breakthrough). This technology could prevent human exposure to toxic chemicals in the industrial workplace, office, medical, and home environments.

Methodology

A vapor dilution and calibration system was built and a test protocol was developed. Commercial sensors were evaluated against analytical and logistic requirements. Additionally, a new type of thin carbon-film sensor was fabricated. The sensors were evaluated for analytical response to chemical vapors (e.g., benzene, CCl_4) and vapor breakthrough test were conducted to test the sensors' abilities to predict adsorbent bed exhaustion.

Progress and Accomplishments

The developments to date include:

1. Available microsensors were surveyed.
2. Two were selected for evaluation along with a sensor designed and built using TRI proprietary technology.
3. The process for fabricating the TRI sensor of the same material as the adsorbent bed (so that it had the same response as the bed) was investigated.
4. A commercial charcoal canister was fitted with a charcoal sensor and the stability was measured.
5. Evaluation of the Phase I data was performed, a final report was prepared, and a patent application and publication are in preparation.

The final results indicate that: 1) the fabrication of a microsensor with the desired low cost and vapor response is possible and 2) use of a charcoal sensor inside the bed results in the ability to predict chemical vapor breakthrough. These results provide the foundation for Phase II development of an optimized sensor and prototype ARD.

Significance

The results of this study show that it is indeed possible to develop a low-cost microsensor whose response is identical to that of the adsorbent bed in which it is inserted. Utilization of this new technology in respirators, air cleaning filters, and other purification systems would greatly increase the effectiveness of maintenance programs, saving potentially millions of dollars in materials. More importantly, inadvertent exposure of personnel in a broad range of occupations to toxic levels of vapors would be minimized.

Publications

U.S. Patent: Application Disclosure in preparation for the "Detection of Vapor Breakthrough in Adsorbent Beds" TRI, 1987

Human Metabolism of Halothane Mechanisms of Toxicity

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Other Occupational Concerns
2 R01 OH00978-07
09/01/80 - 11/30/89
$267,434 ($1,304,780 Cum)

Objectives

We are studying three inter-related aspects of the hepatotoxicity of halothane and related halogenated hydrocarbons: First, the mechanism by which metabolism of halothane, methylene chloride, or ethylene dibromide causes acute hepatic necrosis by rapid damage to essential cellular proteins. Second, the metabolic pathway by which formation of free radical metabolites of these halogenated hydrocarbons leads to peroxidation of cell membrane phospholipids and how some of these peroxidized phospholipids may be converted into potent mediators of inflammation and activators of macrophages. Third, the induction of antibodies in certain individuals against metabolites of halogenated hydrocarbons to which they have been exposed and the mechanism by which a second acute exposure or continued chronic exposure may cause circulating macrophages to attack liver cells. Of particular importance is how these three factors may act in concert.

Methodology

Human liver cytochrome P-450 and its reductase are purified and reconstituted into a phospholipid vesicle system that is capable of metabolism of halothane and other halogenated hydrocarbons. This system is used to study the effect of metabolism on lipid peroxidation and formation of leukotrienes and other lipid-derived mediators of inflammation.

Rat liver cells are isolated and maintained in monolayer culture for our studies of the acute toxicity of halothane, methylene chloride, and ethylene dibromide. These monolayer cell cultures will be used in studies of the binding of specific antibodies to hepatocytes that have metabolized halogenated hydrocarbons and the subsequent attack on these hepatocytes by activated macrophages. The method described by Gillette and Pohl is used to raise antibodies in rabbits against a synthetic antigen formed by coupling a halothane metabolite, trifluoroacetyl chloride, to albumin. We are presently developing techniques to measure blood levels of such antibodies by preparing synthetic haptens from halothane and other halogenated hydrocarbons and attaching them to microspheres.

Progress and Accomplishments

The individual contributions of halothane metabolism, hepatic enzyme induction, and hypoxia to hepatotoxicity were measured in an *in vitro* model of the widely studied rat model for halothane hepatotoxicity. This study shows that halothane metabolism is an essential factor and that hepatotoxicity is not due to decreased hepatic oxygenation, as has been suggested by others.

We have shown that the clearance of leukotriene B_4 by the liver is reduced by an order of magnitude under hypoxic conditions. We have shown that hepatocytes are capable of converting leukotriene A_4 into leukotriene B_4 under hypoxic conditions. We have previously shown that hepatocytes produce the precursor of leukotriene A_4, 5-HPETE, during metabolism of halothane. In the coming year we will attempt to bridge the remaining gap, the conversion of 5-HPETE into leukotriene A_4. This final step would prove that hepatocytes are capable of producing chemoattractants for macrophages and would support the immune mechanism of hepatoxicity that is discussed below.

Significance

Our studies lead to a mechanism of hepatotoxicity that may have general applicability to many toxic chemicals: Following an acute exposure to a chemical, reactive metabolites are produced that bind to proteins in the liver and these modified proteins elicit the production of specific antibodies.

If a second acute or chronic exposure to the chemical occurs, these circulating antibodies will bind to the surface of hepatocytes that have metabolized the chemical and then macrophages, attracted by products of lipid peroxidation and activated by immune complexes, will attack the cells that have bound antibodies and attempt to lyse them. Chronic second exposures that are of too low a level to induce lysis will result in persistent attack by activated macrophages. There is evidence that such a persistent inflammation may initiate hepatic carcinogenesis. This hypothesis would suggest that persons who incur an initial high acute exposure to a toxic chemical should be tested for circulating antibodies against metabolites of this chemical and, if present, be warned against re-exposure.

Publications

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Chromium Distribution and Toxicity in Mammalian Cells

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2 R01 OH01630-05
09/30/83 - 08/31/90
$112,642 ($445,322 Cum)

Objectives

We will conduct a cytophotometric study of the acute/chronic hepatotoxicity of chromium injected into mice. To date, in vivo studies have looked mainly for tumor induction at sites of administration and have characterized chromium-induced malignancies at the level of the whole organ. Our research approach is to use an innovative CAM (computer-assisted microscopy) system to quantify changes in individual cells, as analyzed by cytochemical, immunocyto-chemical and radioautographic techniques. Results of our *in vitro* findings led us to hypothesize that the chromium-sensitive cells are on the order of One Cell Among Many (OCAM), and that the ability to quantify changes in individual cells was pivotal in further analyzing chromium's toxic effects, *in vivo*. Such rare cells cannot be isolated by biochemical assays, being diluted by the "batch" of cells required for such analyses.

To study chromium genotoxicity, *in vivo*, our research strategy will entail a systematic fine tuning of CAM-enhanced analyses, progressing from a "big picture" of the tissue in terms of cell composition, to the cytophotometric and textural analyses of the cell nucleus (see levels of analyses described below). Our research objectives are the following: 1) To study acute toxic effects of chromium administration on the liver, and establish the minimum toxic dose (MTD) of chromium which we can quantify with our CAM. Hepato-toxicity will be described initially in terms of the time- and dose-related increase in apoptotic and necrotic cells and nuclear anomalies following chromium injection, and the evidence of liver regeneration (mitosis) in response to injury. 2) To study effects of chronic chromium administration, based upon the acute MTD studies, focusing on the appearance of tumor cells and the ploidy/nuclearity profile of the injured liver. 3) An initial and on-going objective is to refine and validate the CAM imaging technique for cytophotometric and textural analyses of single cells and nuclei. 4) The ultimate goal is to summarize the CAM-stored database on chromium-induced changes in cells after acute vs. chronic administration, by generating cells archetypes sorted by treatment interval and dosage.

Methodology

The CAM system provides the basis for our research investigations; the light microscope and charge-coupled device to capture the microscopic images, the computer storage to retain those images, and the software to digitally manipulate or extrapolate certain features of an image for analysis based upon algorithms for morphometric and textural parameters. We will use the CAM to analyze Cr(VI) effects on three levels: First Level. Analyze liver tissue according to cell state (i.e., cells that are necrotic, apoptotic, mitotic, neoplastic, or in interphase), and then by cell type (hepatocyte, Kupffer, littoral). Measurements include: cell numbers (percent of total), cell density (nearest neighbor), location or distribution (zonal and periportal region). Second Level. Analyze whole cells by cytophotometric measurements of the whole cell and staining for RNA, protein, and albumin. Cytophotometric measurements include: area, entropy, inertia; digitized images of stained/radio-tagged constituents will provide a relative measure of content. Third Level. Analyze nuclei according to size, i.e., ploidy and binuclearity, and by textural analysis of histochemically stained DNA, RNA, and protein. Use enzymatic removal of selected tissue constituents to enhance textural analysis. Textural analyses include: density, heterogeneity, granularity, and margination.

Progress and Accomplishments

We conducted preliminary studies on CF_1 mice and presently are using BDF_1 mice for our studies. Mice were injected intraperitoneally with 75 mg/kg or 25 mg/kg of sodium dichromate. At the supralethal dose of 75 mg/kg, hepatocytes showed an increase in apoptotic cells over controls, evident 2 hr following injection, and markedly increased at 24 hr following injection. Mice injected with a sublethal dose of 25 mg/kg were sacrificed at daily intervals for a week, and at weekly intervals for four weeks. Preliminary studies indicate an increase in mitosis in hepatocytes, evident at 2 days following chromium injection, and remaining significantly above controls throughout the 4-week period. These studies lend support to the notion that a methodology capable of detecting OCAM, has the sensitivity to detect early and subtle changes, brought about by low doses of toxicants.

Pertaining to the refinement of the CAM software, we have improved the capture of nuclear features, having the capability to measure shape, optical density, and edge detection. We are currently working on a program for the radial segmentation of the nucleus (i.e., "onion rings") for the analysis of non-random optical density patterns, which will be an important textural parameter.

Significance

Preliminary studies indicated that the OCAM (single cell) approach to studying Cr toxicity is valid, i.e., that it is a most sensitive means of detecting the earliest changes *in vivo*; apoptosis may represent the initial cellular response in acute exposure to Cr. Thus far in our MTD studies, mitosis and apoptosis is observed in the first few days after chromium, in the absence of necrosis. Our future investigations will seek to confirm the apoptotic response to sub-lethal doses (MTD) of Cr, and to determine whether repeated administration, to the same MTD, as in chronic exposure, gives rise to tumor cells. If such a correlation is established, then it might prove to be a means of early detection for potential carcinogenic effects of low levels of toxicants in animals, for extrapolation to humans. This is very pertinent to questions of acceptable levels and/or exposure to chromium and other toxicants in industry and the environment, in which it is very difficult to detect injurious changes resulting from acute exposure to the low levels present. On the other hand, with chronic exposure, the presence of tumor cells are more easily detected, but at a point past any alternative means of prevention.

The information obtained at each level of analysis will be useful in dissecting Cr toxicity *in vivo*, due to the precision of measurements capable with the CAM system. In characterizing the tissue-wide response to Cr toxicity, on a per cell basis, we can measure the magnitude, type, distribution, and location of Cr-sensitive cells, and thus, determine if there is a pattern in how and where Cr toxicity is manifested with acute vs. chronic exposure. The significance of the CAM's textural analyses of histochemically stained nucleic acids and protein is that it enables us to analyze the relative content and structure of chromatin and other intranuclear entities (i.e., nucleoli) in Cr-sensitive cells, as defined by selective morphological criteria. Though textural analyses of nuclear constituents are still in their infancy compared to more established biochemical assays, this approach, using selective enzymatic probes and stains, will be useful in describing and localizing the sites of Cr action in liver cells, *in situ*.

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Poison Venters: A Resource for Occupational Health Services

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Other Occupational Concerns
1 R18 OH01981-01A3
02/01/87 - 01/31/89
$156,344 ($156,344 Cum)

Objectives

This project is designed to determine the usefulness of regional poison control centers as both a disseminator of occupational health information and as an occupational disease surveillance mechanism after suitable training and the development of appropriate response protocols.

Methodology

The following activities are included in this project.

1) An occupational health training course will be developed for poison information specialists of a regional poison center and implemented and evaluated for the Cincinnati Drug and Poison Information Center staff; 2) Model protocols will be developed for use by poison center occupational health specialists in responding to requests for information about specific dangerous chemical substances in the workplace. An evaluation of existing information sources, both printed and computerized, will identify the useful toxic substances management and prevention protocols to be used in the development of an uniform information and response system for poison center staff; 3) Occupational health professionals will assist the poison center to maximize the impact of these activities on the dissemination of quality information to the public; 4) Public awareness about the work-relatedness of disease will be enhanced through a public education program and the promotion of the poison center as an occupational health resource; 5) Occupational health data sheets will be designed and used by the poison center for the collection of uniform data on each request and the quality of service provided by the poison center staff will be evaluated by an industrial hygienist and occupational physician; and 6) A program will be developed to evaluate these data for their value as a surveillance method for the detection of occupational disease.

Progress and Accomplishments

The determination of the needs for training of Poison Information Specialists to provide services for occupational or environmental health enquiries was based on results obtained from poison centers responding to a nationwide survey. Responses were received from 30 certified and 68 non-certified centers out of a total 290 centers surveyed. Survey data included annual total number of occupational calls received and staff utilization of available occupational health resources.

Decisions on the format and content of the substance-specific response protocols was determined on the basis of information received from: a) A national survey of 98 poison centers; b) Results of data collected from the Cincinnati Drug and Poison Information Center; and data supplied by the Information Retrieval and Analysis Section of NIOSH. Selection of the fifty substances for the response protocols was based on: a) DPIC case records for either frequently encountered substances or substances which were not clearly reviewed in available resources and b) Frequency of citings from monthly reports of the Informational Retrieval and Analysis Section of NIOSH. Data collection forms and evaluation systems have been developed.

The initial list of substances for which protocols are currently in the process of development are:

Acrylonitrile
Ammonia
Asbestos
Benzene
Carbon monoxide
Chlorine
Creosote
Dichloromethane
Dursban
Ethylene glycol
Ethylene oxide
Fiberglass
Formaldehyde
Hydrochloric acid
Lead
Methyl ethyl ketone
Nitrous oxide
Perchlorethylene
PCBs
Sulfur dioxide
Sulfuric acid
Trichloroethylene
Toluene
Xylene

Significance

The training needs assessment is the first national survey of existing training and training needs of poison center staff for occupational health services. The response protocols being developed will be of considerable use to poison control centers in efficiently and effectively responding to requests for occupational health information.

Improved Personal Protection at Hazardous Waste Sites

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Other Occupational Concerns
5 R01 OH02066-02
09/28/84 - 08/30/87
$120,938 ($238,559 Cum)

Objectives

Goal 1. Investigation of the utility of FTIR spectroscopy performed with an ATR attachment for the acquisition of spectra of hazardous waste materials having a variety of physical and chemical properties.

Goal 2. Investigation of the applicability of forward and reverse search algorithms used in combination with spectral libraries to the identification of components of the hazardous waste mixtures, and the sensitivity of these searches to the presence of minor components of mixtures.

Goal 3. The development of a compatibility testing scheme that utilizes FTIR analysis data, including guidelines on the use of such data to define personal protection strategies.

Methodology

All experiments were performed using a Nicolet 20 SX-1280 FTIR equipped with a 160 megabyte storage module, and a Harrick PLC S1M ATR system. Software development used adaptations to the programs PAIRS and LSFMIX using Fortran 77 and Macro languages.

All samples were prepared from Aldrich (Gold) solvents, or in the case of actual hazardous waste samples, obtained from the U.S. EPA (NEIC).

Progress and Accomplishments

The first step in the achievement of this objective was to define what is meant by "hazardous waste materials":

Matrices:

A. Mixture of hydrocarbons from n-decane through n-eicosane
B. # 2 fuel oil
C. Authentic waste oil from remedial action site
D. Mixture of acrylic enamel paint solvents
E. Synthetic acrylic enamel paint sludge
F. Authentic paint sludge from remedial action site

Compound classes (of target analytes):

1. Chlorinated, cyclic
2. Polychlorinated biphenyl
3. Chlorinated, aliphatic
4. Non-chlorinated, aromatic
5. Phenolic
6. Isocyanate
7. Organophosphate
8. Nitrile

Subsequent investigation found these definitions to be inadequate. Therefore, an investigation into the frequency of occurrence at hazardous waste sites of compounds of significance to health was initiated. Several hazardous waste samples were procured from the U.S. EPA and a variety of other sources. These samples have been subjected to extensive analysis by other methods.

Goals 1-3: A Nicolet 20 SX - 1280 FTIR was acquired, installed in our laboratory, and all personnel trained in its use. Software was installed in the instrument, including forward search routines, the Aldrich medium and high resolution spectral libraries, the Boolean logic program GRAB, and the artificial intelligence program PAIRS. Goal 1: Progress into the investigation of the applicability of ATR for spectral acquisition is complete. Questions of ATR versus transmission cells center on the questions of shifts of absorbance peak maxima and absorbance ratios. This project is central to our present effort.

Goal 2: Investigation of forward search routines was completed. Investigation of the program PAIRS for the identification of functional groups of principal constituents of simulated hazardous waste mixtures was completed. Application of PAIRS to the identification of the principal components of simulated hazardous waste mixtures was completed. Development of the program PAWMI was completed. The programs LSF and LSFMIX, as well as a variation on these programs written in our laboratory, were investigated for spectral curve fitting and quantitation, and found to be inapplicable. However, the program DECON was found to be useful to deconvolve overlapping peaks. An automatic rule generator and optimizer for PAWMI is being developed. The application of the automated version of PAWMI to spectra that have been deconvoluted will complete this objective.

Goal 3: The goal of incorporating these findings into a hazardous waste site personal protection strategy has not been addressed.

Significance

The above progress represents a very substantial portion of the work that can be accomplished in the laboratory towards investigating the utility of FTIR for the improvement of personal protection strategies at hazardous waste sites. The advances have been accomplished in analytical chemistry that will allow the industrial hygiene advances, but not without actual field testing. Proposals have been submitted to perform field testing of the method, and application of the findings to industrial hygiene during that field trial.

Publications

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Polyimide Sorbents for Airborne Toxic and Carcinogens

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Other Occupational Concerns
5 R01 OH02108-02
01/01/86 - 06/30/88
$148,195 ($289,350 Cum)

Objectives

The long-term thrust of this research has been to characterize the performance of four promising polyimide sorbents which can be used for collecting toxic, mutagenic, or carcinogenic chemicals from workplace, indoor or outdoor air, and performing chemical and biological assays on the collected samples.

The effect to which the air sampling process maintains ideality with the polyimide sorbents was studied during the past year. Laboratory experiments were conducted to gain insight into the fundamental and practical understanding of those factors producing non-ideal behavior. Specifically, the following questions were addressed: (a) Does air humidity alter the breakthrough volumes of analytes for a sorbent? (b) Are breakthrough volumes for individual analytes altered by competition between analytes in a mixture for sorption sites? Are there a variety of adsorption mechanisms operative on the sorbent surface and are they chemical class-related? The recovery of analytes using thermal and supercritical fluid CO_2 conditions was also studied.

Methodology

For a pure physical adsorption mechanism at concentrations yielding linear adsorption isotherms, the retention of the components of a mixture should be ideally independent of each other and equal to the retention of the pure compound under the same conditions of temperature, pressure, etc. The extent to which the air sampling process deviates from this ideal case has practical implications for sorbents. Laboratory experiments were conducted to gain insight into those factors which may produce non-ideal behavior.

Initially, experiments were conducted with six chemicals to probe four types of molecular interactions which might occur during adsorption on each polyimide and Tenax GC. There were: (1) water (Class AB, strong hydrogen bonding), (2) ethanol (Class AB, weaker hydrogen bonding, electron donor properties), (3) 2-butanone and nitromethane (Class B, no active hydrogen bonding, electron donor properties), and (4) hexane and benzene (Class N, no active hydrogen bonding. Van der Waals forces and π-π forces, respectively).

Using a deuterated tracer pulse technique with mass spectrometric detection, the method of Parcher (1-4) was modified. A mixture of chemicals were introduced in a continuous gas stream (frontal) into the sorbent bed, and at periodic intervals (-10, 0, 10, 20, 30 min; at 0 min the frontal stream was begun) discrete injections (-5 μg) of the corresponding deuterated chemical into the frontal stream were made. The percent deviation in the breakthrough volume (BV) of the deuterated chemical was determined relative to no "polluted frontal stream."

Radiolabeled γ-BHC, hexachlorobiphenyl, anthracene, and parathion, representing the chemical classes of chlorinated aliphatic and aromatic compounds, polynuclear aromatics, and organo-phosphate pesticides, respectively, were used to demonstrate the ability of supercritical carbon dioxide to desorb these chemicals from polyimides and Tenax-GC.

Progress and Accomplishments

From the data studied, the following observations were evident:

(a) a frontal rate of 80-100 nmoles/min/chemical begins to exceed the capacity of PI-109 (bed dimensions: 2 mm x 10 cm) for each analyte (Expt. 1,13,14):

(b) elevating the frontal rate ten-fold for:

    (1) hexane had a small effect on its own, benzene's, and nitromethane's BV's, and a much larger decrease for ethanol and butanone, suggesting different types of adsorption sites (Expt. 2);
    (2) ethanol had most pronounced decreases in BV for ethanol and butanone (Expt. 3);
    (3) benzene produced similar BV changes as ethanol (Expt. 4);
    (4) nitromethane exhibited pronounced decreases in BV for itself, ethanol and butanone(Expt.5)
    (5) butanone produced significant decreases in BVs for ethanol, benzene, nitromethane and itself (Expt. 6).

(c) presence of humidity in the frontal stream significantly decreased the BV's for the "pulsed chemicals" in the absence of organics in the frontal stream, whereas, the organics present in the frontal stream tended to offset this decrease suggesting that water vapor introduces another sorption mechanism when it is present in the frontal stream.

These results suggest that the sorptive capacity is a function of several sorption mechanisms (analyte-sorbent) at the molecular level, as it may be predicted by the various chemical functionalities present on the sorbent surface. The deuterated pulse tracer technique was shown to be useful for probing into these mechanisms of action under practical dynamic field sampling conditions, and providing answers to the original questions on effects of humidity and analyte composition on BVs. Additional data of this nature has been acquired for Tebax GC and other polyimides. Toxic chemicals of interest to NIOSH are also being studied.

All test compounds were recovered in excessive of 90% on Tenax GC and except for anthracene, greater than 80% on the polyimides. The identity of the desorbed compounds was verified using TLC and mass spectrometry. These results were compared to thermal desorption from Tenax for the same chemicals where, even under the more favorable conditions for thermal desorption, hexachlorobiphenyl and parathion was well below 50%. An interface is now being designed to recover analytes from sorbents by supercritical CO_2 and their transfer to analytical separation systems for analysis.

Significance

The deuterated pulse tracer technique yields extremely rich information and is useful in probing the mechanisms of analyte-sorbent interaction and competition under practical sampling conditions. This technique could serve as a standard technique for assessing any sorbent under consideration for sampling of organic vapors from air.

Recovery of analytes using supercritical fluid CP_2 allows the range to be extended to semivolatile chemicals which are not recoverable by thermal means. Also, conditions are mild (40°C) permitting analysis of labile, polar chemicals while minimizing potential artifacts attributable to thermal desorption. A method based upon supercritical fluid CO_2 should permit more sensitive analyses than can be achieved with liquid desorption.

Publications

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Plasma Proteins: Markers of Chemical Exposure

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5 R01 OH02149-02
01/01/86 - 12/31/88
$ 92,030 ($193,589 Cum)

Objectives

A significant number of people are exposed to a variety of chemicals at work sites which may be responsible for toxic manifestations. Such exposure(s) can bring about changes in plasma proteins in terms of their function, concentration or covalent modification. These changes, besides being responsible for toxic effects, can also be used as markers of chemical exposure. The intent of this research is to develop method(s) which can identify the changes in plasma proteins caused by chemical exposure.

Methodology

Effects of chemical exposure on plasma proteins were studied in terms of their biological activity, concentration, and covalent modification. These changes were measured using bio- and immunoassays, electrophoretic and chromatographic techniques. Covalent modification was further characterized by peptide mapping, compositional and sequential amino acid analysis. The structure of the modified amino acid(s) will be determined by spectral techniques and further confirmed by independent synthesis.

Progress and Accomplishments

In an earlier study we have shown that plasma α_1-proteinase inhibitor (α_1-PI) is inactivated by aldehydes found in the cigarette smoke, acrolein being the most potent one. To elucidate the mechanism by which acrolein inactivates α_1-PI, samples of α_1-PI were incubated with increasing concentrations of acrolein at 37°C for 2h. The proteinase inhibitory capacity was assayed against elastase, and the amino acid compositions was determined by amino acid analyses. The loss of the elastase inhibitory capacity (EIC) correlates well with the disappearance (hense modification) of the lysine and histidine residues, which in turn is proportionally dependent on the concentration of acrolein used. More than 90% of EIC was lost when 23 lysine and 8 histidine residues were modified. In addition, a new peak was observed between histidine and arginine upon amino acid analysis of acrolein treated α_1-PI. The same peak was observed when model compounds (N-acetylysine or polylysine, M.W. 35,000) were reacted with acrolein and subsequently processed for amino acid analysis. This new compound is most likely an adduct of acrolein with lysine residues of the protein. We are in the process of purifying this compound in sizable amount which will be used for structure elucidation by spectroscopic techniques.

Besides aldehydes found in cigarette smoke, chemicals of industrial importance also inactivate α-PI. We have demonstrated the synergistic inactivation of α_1-PI by mixing aldehydes (acrolein or pyruvic aldehyde) with industrial chemicals (styrene oxide or 1,2-DCE and vice-versa). The data obtained from these studies suggest that smokers exposed to chemicals in industry may be more prone to lung emphysema due to synergistic inactivation of α_1-PI by chemicals and cigarette smoke components.

We have also explored the possibility of using glutathione S-transferase (GST) of red blood cells as a marker of chemical exposure. These studies were conducted with purified (GST) from human erythrocytes as well as in red blood cells. Acrolein, styrene, propylene and ethylene oxides, 1,2-dibromo and dichlono-ethanes 1,2-DBE and 1,2-DCE) caused a dose dependent inhibition in the activity of GST using 4-chloro-2,6-dinitrobenzene as a substrate. This raised the possibility of using GST as a marker of chemical exposure.

To study the binding of plasma proteins with industrial chemicals, rats were exposed by gavage to ^{14}C labeled benezene, 1,2-DBE and 1,2-DCE for twelve days (25 mg/kg in mineral oil), blood was drawn on thirteenth day through exterior vena cava and separated into plasma. After dialysis, the radioactivity in the plasma was measured. 1,2-DCE shows maximum binding with plasma proteins followed by 1,2-DBE. Minimum binding of plasma proteins was observed with benzene. The plasma was further separated by size-exclusion high-performance liquid chromatography (SEHPLC) and the radioactivity was found to be exclusively associated with proteins of mol. wt. 65KD. This peak (observed at 280 nm) was further confirmed to contain mainly albumin by immunodiffusion and reversed phase high performance liquid chromatography. Attempts are being made to identify the peptide fragment as well as amino acids to which radioactivity is associated.

Significance

Although the present study is limited to *in vitro* studies with human plasma, and *in vivo* studies in rats, the experiments could be extended to monitor occupationally exposed populations to these or other chemicals. Correlation between the changes in plasma proteins and the medical histories of the occupationally exposed individuals can eventually be used for medical surveillance.

Publications

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Gan JC, Ansari GAS: Plausible Mechanism of Inactivation of a_1-Proteinase Inhibitor by Acrolein. Res Commun Chem Path Pharmacol 55:419-422, 1987

Ansari GAS, Singh SV, Gan JC, Awasthi YC: Human Erythrocyte Glutatione S-Transferase: A Possible Marker of Chemical Exposure. Toxicol. Lett. 37:57-62, 1987.

Ansari GAS, Gan JC, Barton BK: *In Vitro* Inactivation of Plasma a_1-Proteinase Inhibitor by Exposides and 1,2-Dihaloethanes. Arch Environ Contam Toxicol in press

Ansari GAS, Gan JC, Barton BK: Synergistic Inactivation of Plasma a_1-Proteinase Inhibitor by Aldehydes of Cigarette Smoke with Styrene Oxide and 1,2-Dichloroethane. Arch Environ Contam Toxicol in press

Environmental Toxicity of Isocyanates

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Other Occupational Concerns
5 R01 OH02214-02
02/01/87 - 01/31/90
$124,677 ($255,731 Cum)

Objectives

The overall goal of the proposed research is to gain an understanding of the molecular events involved in the various toxic responses to isocyanates. These responses include sensory and pulmonary irritation, sensitization and chronic impairment of pulmonary function. The three specific aims are to identify *in vivo* sites of modification by inhaled isoyanates, to measure the hydrolysis rates of isocyanates under normal atmospheric conditions, and to determine the specificity of the isocyanates toward protein receptors.

Methodology

The measurement of the fate of inhaled isocyanates is being accomplished by exposing animals to atmospheres containing known concentrations of radioactively labeled isocyanates. Isocyanates being tested are toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI) and methyl isocyanate (MIC). Atmospheres are generated using procedures previously described by this group. Tissues from the respiratory tract, as well as all other major organs and body fluids are collected following the controlled exposures. The tissues are subjected to biochemical analysis using extraction, electrophoretic and chromatographic methods, and to histological analysis using standard staining and autoradiographic techniques to identify the form and location of the radio-label. The physiologic fluids are similarly fractionated to analyze for soluble macromolecular targets as well as metabolic reaction products of isocyanates. To characterize the state of the isocyanate under atmospheric conditions, isocyanate hydrolysis will be measured under controlled aqueous conditions using NMR spectroscopy. Using this technique, the structure and rate of formation and disappearance of all products and intermediates can be determined. The identification of the specific reaction sites of isocyanates on protein targets will be investigated using standard protein chemistry methods. *In vivo* labeled proteins will be purified to homogeneity and identified by automated Edman sequencing. Location of the labeled amino acid in the sequence will be accomplished by digestion with proteolytic enzymes and fractionation of peptides by HPLC. The radio-labeled fragment(s) will be subjected to Edman sequencing and the modified amino acid will be identified by comparison to synthetic model compounds. *In vitro* labeled targets will be investigated in a similar manner after complete characterization of the specificity of the reaction under defined conditions.

Progress and Accomplishments

Major emphasis has been placed on the analysis of the fate of inhaled isocyanates. Progress has been made on the analysis of the uptake, distribution and form of two isocyanates: toluene diisocyanate and methyl isocyanate. The following is a brief summary of the current results supported by this grant.

Toluene diisocyanate shows a linear uptake in the blood during the first 150 minutes of a 300 minute continuous exposure to 0.2 ppm ^{14}C-TDI vapor. After the initial uptake, a steady state appears to be established. The label is covalently attached to the trachea and lung and transiently accumulates in the kidney and liver. Though present in other organs, the level of radioactivity can be accounted for by the quantity of blood present in those organs. Analysis of the blood shows that the radioactivity is primarily associated with a single protein and that no significant label is found associated with molecules with molecular weights at or below 30,000 KDal. The nature of the Accumulated labeled material in the liver and kidney is under investigation.

Methyl isocyanate shows a linear uptake of radioactivity during the entire exposure period. Three different concentrations were investigated: 0.5, 5 and 15 ppm. Analysis of animals at times post exposure showed that the amount of label in the blood continued to increase for a period of an hour after termination of the exposure, and that the label in the body fluids, including blood, oile and urine, followed parallel patterns of clearance with the majority of the label removed from the system within 24 hours post exposures. Histological analysis of tissue showed accumulation of the label in areas similar to those found with TDI; however, after 24 hours, the label is significantly diminished in the case of MIC. Preliminary analysis of the blood shows that there is a significant level of low molecular weight (1000 daltons) radioactive material in the postexposure samples. This material diminishes with time. Quantitative assessment of these findings is being completed.

Preliminary analysis of the 77 kDal *in vivo* labeled serum protein indicates that it is an "albumin-like" protein. Sequence analysis indicates that it is homologous but not identical to serum albumin. *In vitro* studies have been initiated to determine the specificity of modification of true serum albumin by isocyanates. Preliminary studies show that serum albumin binding characteristics are latered by the specific interaction with toluene diisocyanate. Currently, antibodies are being made toward this specific conjugate.

Significance

Identification of the specific *in vivo* targets of inhaled isocyanates will enable development of appropriate radioimunoassays for use in the analysis of blood or urine samples from workers to determine the extent of their exposure to isocyanates. The application of the exposure protocol used in these studies may provide new information to define different threshold events in the exposure to isocyanates. These include a true definition of the threshold for tissue damage, for sensitization, and for appearance of circulating modified macromolecules. Results of these studies will identify *in vivo* reactions of airborne isocyanates and provide methods for quantitative evaluation of isocyanate exposures. Conclusions drawn from these studies may be used in evaluating mechanisms involving exposure to other reactive gases.

Dose/Response for Occupational Styrene Exposures

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Other Occupational Concerns
5 R01 OH02221-02
9/29/86 - 09/28/88
$135,524 ($259,214 Cum)

Objectives

This study is investigating the linkages between exposure, absorbed dose, and genotoxic response resulting from occupational exposure to styrene in the reinforced-plastics industry. A longitudinal study is underway in which 48 workers in a single reinforced-plastics facility are being monitored. Each individual's airborne exposure is monitored 8-10 times (shift-long sampling), his/her blood is collected four times, and his/her exhaled air is collected 24-30 times over a 12-month period. The primary objective of the exposure assessment is to classify workers into uniform-exposure groups according to the means and variances of their (within) exposure distributions. A secondary objective is to correlate the levels of styrene in the mixed exhaled air, as measured by a new technique, with the levels of styrene in blood collected at the same time. If the correlation is good, this will allow the individual workers' distribution of short-term burdens of styrene to be inferred from the non-invasive and simple method for exhaled-air determination. Thus, the exposure assessment should provide information concerning both average and transient exposure/dose conditions experienced by each member of the cohort.

Various measures of genotoxic response are measured in the blood samples. These include: sister-chromatic exchanges, micronuclei, and DNA adducts in the peripheral lymphocytes, and hemoglobin adducts in the red blood cells. The overall objective of the investigation is to determine the extent to which fluctuations in the transient dose of styrene (at a given mean exposure) influence the levels of these various genotoxic markers. This will provide information to ascertain the possible need for a short-term exposure limit for styrene. Secondary objectives include correlating the various markers with each other in a pool of common blood samples and in determining the dose/response for styrene in the low-exposure region of 5-20 ppm (mean exposure) where little information currently exists.

A separate aspect of the investigation concerns the application of a pharmacokinetic model for styrene, derived under constant-exposure conditions, to the situation typical of occupational exposures where air levels vary considerably over time. The input of the model is a lognormally-distributed, autocorrelated series of exposure concentrations while the output consists of the resulting concentrations of styrene in the central and peripheral compartments. A portion of the field study gathered short-term exposure data and blood concentrations at intervals of 15 min to validate the model.

Methodology

Exposures are being measured with passive monitors employing cocoanut carbon as the collector. Measurement of styrene in the exhaled air employs a new device in which the individual forcibly exhales through a commercial tube containing 200 mg of cocoanut carbon. The samples are stable almost indefinitely prior to analysis via gas chromatography. Blood styrene is measured via the head space technique using standard addition and gas chromatography. The method for measuring DNA adducts is a modification of the ^{32}P post-labeling technique developed by Randerath and coworkers. Modifications were required to increase the sensitivity sufficiently to allow adducts to be measured in the human lymphocytes. A HPLC method is being developed for detecting hemoglobin adducts. Sister-chromatid exchanges and micronuclei are measured according to standard published procedures.

Progress and Accomplishments

The full cohort of workers was assembled and has been monitored on four separate occasions over a 4-month period; blood was gathered on two of the four surveys. Preliminary indications are that exposure to styrene among the cohort ranges from nondetected to 50 ppm mean concentration. It is also clear that some of the exposed individuals have much greater peak exposures at a given mean level than others in the cohort.

Results indicate that there is a good correlation between concentrations of styrene in the blood and the exhaled air as measured by the new technique. Preliminary trials with two volunteers also indicate that there is a good correlation between exhaled air concentrations and both blood and exposure air concentration measured at 15-min intervals for 4 hrs. This suggests that the use of the exhaled-air method to infer transient styrene dose is justified.

Significant progress was made in modifying and applying the ^{32}P post-labeling technique to measure styrene/styrene-oxide DNA adducts. Five DNA adducts have been detected in both in vitro modified nucleic acids, DNA and 9L cells, and in lymphocytes from the exposed workers.

An algorithm for generating a lognormally-distributed autocorrelated series of 15-min air concentrations has been developed and tested with the 2 compartment open pharmacokinetic model. Results of the simulation are consistent with preliminary blood-styrene data.

Sister chromatid exchanges and micronuclei have been measured in workers lymphocytes from the two blood samplings conducted thus far. It is premature at this time to tell whether there is a dose-related increase in the numbers of these two markers among the cohort.

A method is being developed for measuring hemoglobin adducts of styrene in the blood of exposed workers. The method is based upon an HPLC determination of the adducted N-terminal valine of the hemoglobin chain via a modified Edman degradation. The adducts have been synthesized and chemically characterized and HPLC conditions have been developed. While adducts have been measured in styrene-oxide modified blood samples in vitro additional cleanup steps are required to allow quantitation at the levels encountered in the workers' blood. Additional development of the procedure is proceeding along this line.

Significance

This project represents one of the first comprehensive applications of biochemical epidemiology to an occupational cohort. The extensive exposure assessment performed longitudinally will allow the variation in exposure to be partitioned into within- and between-worker components to allow workers to be properly classified. It should also be possible to determine whether any of the genotoxic markers appear to be influenced disproportionally by high transient (peak) exposures to styrene; this was the original hypothesis proposed for testing. The development and application of methods for detecting adducts of DNA and hemoglobin will, if successful, produce important new information which was not part of the original proposal. The new method for measuring styrene in the exhaled air is the first simple method reported which allows the sample to be stored for long periods prior to analysis. This method can probably be applied to a wide variety of organic solvent vapors.

Publications

Rappaport SM, Spear RC: Physiological Dampening of Exposure Variability During Brief Periods. Ann Occup Hyg, in press

Artificial Intelligence Occupational History

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Other Occupational Concerns
1 R01 OH02288-01
05/01/87 - 04/30/89
$106,294 ($106,294 Cum)

Objectives

The recognition of occupational lung disease requires the association between clinical findings and environmental exposures. This project will develop an artificial intelligence occupational history system (AIOHS) to acquire information concerning jobs, industries, and exposures (agents) as well as clinical information and to integrate this with a knowledge database to suggest diagnosis of occupational lung disease. The AIOHS will selectively request information as needed to achieve diagnoses. The AIOHS will be validated by comparison to experienced occupational health professionals and its acceptability will be assessed by extensive field trials.

"The AIOHS will be microcomputer based to enhance its general utility. The system will employ existing artificial intelligence methods for symbolic processing and predicate logic."

The AIOHS should be useful for several purposes:

1. Diagnosis and education for occupational lung disease;
2. Occupational health surveillance;
3. Detection of confounders in epidemiologic studies; and
4. As a demonstration of the ability of artificial intelligence in occupational health.

Fast-GC for Industrial Hygiene Monitoring/Analysis

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Other Occupational Concerns
5 R01 OH02303-02
09/29/86 - 09/28/88
$95,360 ($211,263 Cum)

Summary

The development of a thermally desorbable miniature passive dosimeter (MPD) for very high sensitivity STEL monitoring of organic vapors has been completed by our research group. Given an analysis time of 15-30 minutes per sample, plus quality control assays, a single series of STEL samples from one worker would tie up a gas chromatograph (GC) for a full day. Therefore, there is a need for a rapid GC method of analysis for organic vapor samples collected on passive dosimeters.

The use of GC for the real-time analysis of vapors is also severely limited by the analysis time required and the lack of sensitivity of the method. For example, for multiple components, such as at a refinery, each GC analysis will take 15-30 mins, sensitivity will be significantly degraded, and the utility of the GC method for area monitoring/alarm will be seriously compromised. Therefore, there is a need for a rapid, sensitive GC method of analysis for organic samples collected as vapors (as with a plant area monitor/alarm).

GC is also used for the analysis of CS_2 eluates of adsorbent tubes. These analyses generally takes 10-30 minutes per sample. A method that would permit the analysis of CS_2 solutions in 10 seconds would save significant laboratory resources.

Recently, a high speed GC system has been described by our research group that was demonstrated to be capable of analyzing a nine-component test mixture of organic compounds in just over 6 seconds. This is an improvement in analysis speed when compared to conventional GC of 10^2 times. Along with the improvement in speed, an expected improvement in sensitivity on the order of 10 times is expected. Therefore, this proposal is aimed at testing the following hypotheses:

1. Vapor samples collected on passive dosimeters can be thermally desorbed/analyzed using the fast GC system in 10 seconds or less with no degradation of performance when compared to conventional GC methods.
2. Air samples can be directly analyzed within 10 secs at sensitivities significantly higher than those achievable using present methods.
3. CS_2 solutions of target analytes can be analyzed in 10 seconds.

Self-Training, Self-Optimizing Infrared Expert System

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1 R01 OH02404-01
09/29/87 - 09/28/89
$123,887 ($123,887 Cum)

Summary

With the advent of Fourier transform infrared (FTIR) instrumentation, IR techniques have been increasing application in the area of environmental analysis. Environmental analysis applications of FTIR include the characterization of hazardous waste mixtures and the speciation and quantitation of airborne vapors and gases both in ambient air, vehicular emissions and the workplace.

The most commonly applied method of spectral identification uses forward searching of infrared spectral libraries. This method succeeds where the compound and the resulting spectrum is pure, or where the spectrum of a commercial mixture is available. The method fails in the case of the spectra of mixtures that are not stored as mixtures in the library. Methods of identifying the compounds in the spectra of mixtures, especially in environmental mixtures, have been pioneered by our group, based on the work of Woodruff, et. al. and Herget, et. al. This approach was achieved through the use of a three level rule structure for each peak, giving increasing "goodness" scores for each peak in successively narrower frequency windows. At the heart of the programs that are used to accomplish either compound or compound class identification are PAIRS and PAWMI. This proposal is aimed at research that will enable these programs to self-train and self-optimize. Specifically, the hypothesis is: A self-training, self-optimizing expert system can be developed to identify the components of mixtures of environmental significance using infrared spectrocopy.

1. Optimization of weighing factors for peak goodness based on frequency of occurrence of peaks in each wavenumber window for given training sets.
2. Optimization of peak window widths for rules containing three peak position windows for each expected absorption.
3. Incorporation of a program to deconvolute overlapping peaks.
4. Incorporation of the automated rule generator, the automated rule optimizer, the deconvolution and peak picking programs, and the mixture interpretation program into one expert system applicable to both condensed and vapor phase species.

Each of the above specific aims involves basic research into the use of expert systems for spectroscopic analysis. This research will be conducted using appropriate training sets of environmental significance.

Halogenated Hydrocarbon Toxicity in Proximal Tubules

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Other Occupational Concerns
1 R01 OH02417-01
09/01/87 - 08/31/90
$92,802 ($92,802 Cum)

Summary

Chloroform and carbon tetracholoride are environmental and occupational contaminants with documented nephrotoxic potential in humans and laboratory animals. The primary site of kidney damage produced by these solvents is the proximal tubule. Although a significant amount of information has been obtained regarding the hepatotoxicity of these halogenated hydrocarbons, comparatively less is known concerning their nephrotoxicity, especially their action on proximal tubule cells. The proposed research will investigate the toxicity of chloroform and carbon tetrachloride using isolated human and rabbit proximal tubule segments *in vitro*. Proximal tubule segments will be isolated from human and rabbit kidneys using purely mechanical techniques that do not require the use of digestive enzymes. The first series of studies will examine and compare the concentration and time dependence of chloroform- and carbon tetrachloride-induced damage to human and rabbit proximal tubules *in vitro*. Other experiments will test whether those factors that are known to alter the toxicity of these halogenated hydrocarbons to liver also affect the severity of cellular damage to tubules. These studies will utilize various procedures to modulate tubular cytochrome P-450 activity, intracellular cysteine and glutathione concentrations and lipid peroxidation in order to correlate changes in these parameters with halogenated hydrocarbon toxicity. The proposed research will combine cellular toxicology and biology methodologies to describe the effects of these halogenated hydrocarbons on the structure and function of human and rabbit proximal tubule cells *in vitro*. The proposed research will: (1) add greatly to our knowledge of the biochemistry of human proximal tubule cells, (2) improve our understanding of the mechanisms of chloroform and carbon tetrachloride nephrotoxicity, and (3) provide a basis for further research leading to meaningful predictive *in vitro* toxicity studies using proximal tubule segments.

Effects of Job Hazards, Health Incentives on Absenteeism

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Other Occupational Concerns
1 R01 OH02586-01
09/29/87 - 09/28/88
$28,147 ($28,147 Cum)

Objectives

Use will be made of the University of Michigan's 1972-73 and 1977 waves of the Quality of Employment Surveys (QES73, QES77) of roughly 1500 randomly selected full-time U.S. employees. (1) The QES77 data tape contains information on 17 possible job hazards including exposure to dangerous chemicals, fire or shock, extremes of temperature, noise and others and 34 possible injuries or illnesses that in the respondent's view were caused or made more severe by the job. The 17 possible hazards and the 34 illnesses and injuries will be ranked based on their relationship to number of days absent. (2) The QES73 data tape contains information on respondents' absences within the past two weeks. Treating absence responses as a dependent variable, Tobit regressions, which are especially designed for truncated, non-normally distributed dependent variables, will be constructed to assess the importance of a variety of independent variables in explaining absenteeism. Independent variables will include: occupational mortality rates derived from the principal investigator's NSF-funded research, wages, availability of sick leave, moonlighting, local unemployment, work hours schedule, unionization, marital and family status, spouse's income, age, race, sex, education, commuting distance, amount of overtime, job satisfaction, subjective evaluation of health, presence of back pain, sleep problems, feelings of fatigue and nervousness, obesity, and smoking. Drinking behavior will also be assessed with unique information on the average number of drinks per month for the respondent. (3) Treating occupational mortality rates and the probability of sustaining a work-related injury as measures of overall job hazards, an assessment will be made of the relative importance of job hazards in explaining absenteeism holding constant other independent variables mentioned in (2). (4) The Tobit statistical model which is much better suited to explaining days absent than multiple regression will be discussed with advantages and disadvantages. A method for assessing the importance of any covariate using statistical significance and elasticity will be explained and examples provided.

Methodology

Three methods will be used: (1) Percentages of persons complaining of various job hazards and injuries by occupation will be tabulated. (2) Tobit regressions will be used to explain fluctuations in self-reported absences with the independent variables mentioned in objectives 2 and 3. (3) The research will consider an alternative method for assessing the importance of an independent variable in a regression which will make use of elasticity -- a concept borrowed from economics.

Significance

The results should be valuable to business executives and policy makers who would like to reduce absenteeism. For an individual firm, a reduction of job hazards might entail resources to be drawn from wages so that workers can now work in a safer environment but receive less pay. But the reduction in the hazard might result in an increase in productivity, from, for example, the reduction in absenteeism. An increase in productivity could result in more profits available for higher wages. It, therefore, becomes necessary to identify particular job hazards that would be the cheapest to reduce and provide the business with the greatest benefit in reduced absenteeism. The cost of hazard reduction will not be addressed in the proposed research. A possible benefit due to the reduction of 17 hazards will be addressed.

Peripheral Markers of Muscarinic Receptors

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Other Occupational Concerns
5 K01 OH00054-02
09/29/86 - 09/28/89
$32,400 ($64,800 Cum)

Objectives

This project will test the hypothesis that muscarinic receptors on circulating lymphocytes could represent a marker of the same receptors in the CNS, the lung and other solid tissues and that alterations of muscarinic receptors in such tissues due to environmental or genetic factors, to pharmacological treatment or to chemical exposure, will be detected by measuring receptor density, affinity and function in lymphocytes.

Methodology

In an initial series of experiments, attempts were made to utilize ^{3}H-methyl scopolamine to label muscarinic receptors on lymphocytes. It was found, however, that this ligand can bind in a specific and saturable manner to glass fiber filters and that this binding site had some of the pharmacological characteristics of muscarinic receptors. Although specific binding to filters was low in relation to the total amount of radioactivity, and may not play a relevant role when tissues with a high content of muscarinic receptors are being assayed, for the quantification of muscarinic receptors in tissues with low receptor concentration, filter binding could be a source of artifacts. Principally for this reason, studies on muscarinic receptors on lymphocytes were then conducted using ^{3}H-quinuclidinyl benzilate (QNB), whose specific binding to glass fiber filters is negligible. These studies were conducted with a mixed population of lymphocytes isolated from rat spleen. The spleen was chosen for this initial characterization since a larger amount of cells can be obtained from one animal (as compared to isolating lymphocytes from blood).

Progress and Accomplishments

Protein linearity studies and saturation binding experiments were initially conducted. An extensive series of experiments were performed to characterize the pharmacological specificity of this binding site for ^{3}H-QNB, and a large number of cholinergic and noncholinergic drugs were tested for their ability to inhibit specific binding of ^{3}H-QNB. These studies indicated that the binding site for ^{3}H-QNB on spleen lymphocytes has for the most part, the characteristics of a muscarinic cholinergic binding site, with, however, a much lower affinity for muscarinic compounds compared to other tissues. Studies in progress are characterizing muscarinic cholinergic receptors in circulating lymphocytes in order to confirm the results obtained with lymphocytes isolated from spleen.

Significance

These studies are providing relevant data on the characteristics of muscarinic receptors on lymphocytes and represent the basis for testing the hypothesis that these receptors could be used as markers of the same receptors in solid tissues. This knowledge will be useful for further studies involving the assessment of the effect of exposure to humans to chemicals affecting the nervous system and other systems.

Publications

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Highway Maintenance Cohort Mortality Study

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Other Occupational Concerns
5 K01 OH00055-02
09/29/86 - 09/28/89
$32,400 ($64,800 Cum)

Objectives

A reported leukemia excess in Wheaton, Minnesota, was investigated by the Minnesota Department of Health (MDH) in 1979-1980. This investigation revealed that five out of six men with leukemia had been employed as highway maintenance workers (HMWs). The leukemia excess could not be associated with job histories, farming practices, or other personal data. The MDH concluded that a large-scale study of all highway maintenance workers was necessary. This conclusion was based on three factors: 1) The leukemia occurrence among highway workers in Wheaton was not, in and of itself, evidence of increased risk among these workers. Their work experience was not unique; more importantly, statistically unusual clusters of leukemia are frequently reported in the scientific literature; 2) Highway maintenance work may involve exposure to a variety of potentially harmful materials, and disease risks other than leukemia may be important; and 3) The number of past and current highway maintenance workers employed by government (city, county, and state) in the U.S. probably exceeds 500,000.

Methodology

A retrospective cohort mortality study of highway maintenance worker mortality was initiated in April 1985. Through various record sources, all men were identified who ever worked in highway maintenance for the Minnesota Department of Transportation (MNDOT) between 1945 and 1984. Standardized mortality ratios were computed using computer programs developed by the MDH for this purpose. The indirect method of standardization was used. The comparison population was other Minnesotans of the same age, sex, and race.

Progress and Accomplishments

A total of 4849 workers were included in this study based on the 3 major eligibility criteria: 1) male; 2) worked at least one year as a HMW; and 3) employed by the MNDOT anytime between 1945 and 1984.

The total number of deaths from all causes was 1530, while 1676 deaths were expected (SMR = 91; $p < .01$). There was no trend with increasing duration of employment. The 9 percent deficit was accounted for by lowered mortality among all three major causes of death: heart disease, cancer, and cerebrovascular disease.

There were 278 cancer deaths overall, which was 17 percent fewer than expected (SMR = 83; $p < .01$). There was no evidence of increasing risk with increasing duration of employment. The deficit in overall cancer deaths was affected by the observed deficits in several of the most common types of cancer, including lung cancers and gastrointestinal cancers.

No overall elevation in mortality was noted for the category that included leukemias, Hodgkin's disease, lymphomas, and multiple myelomas (SMR = 95). Within this category, however, a slightly greater than expected number of leukemia deaths was found (SMR = 107). All of the 17 observed leukemia deaths occurred during the period 1965-1984. None were observed during 1945-1964 time period, although 5 were expected. A statistically significant elevation in leukemia (SMR = 425; $p < .01$) occurred among workers with 30-39 years of work experience and who started work between 1900 and 1944. Excess leukemia risk was found for both urban and rural workers. Overall, there were 19 cancers of the urinary system (SMR = 92), and there was no trend with increasing duration of employment or year started. There was a greater than expected number of deaths for those who died 40-49 years after the start of employment (SMR = 292; $p < .05$).

Based on 17 deaths, the SMR for diseases of the genito-urinary system was 77. Based on 8 deaths, there was no overall increase in deaths from chronic renal failure (SMR = 110). Risk did not increase with increasing duration of employment. There were 3 deaths, however, that occurred among men who had started work at least 50 years before their deaths, a number significantly greater than expected (SMR = 676; p < .05).

Ninety-seven deaths were due to accidental causes (SMR = 121). Since transportation accidents were considered to be a category of special interest prior to the study, these findings were evaluated in greater detail. Transportation accidents involved any accidental death involving a motorized method of conveyance (car, truck, motorcycle, boat, snowmobile, etc.). Overall, there were 53 such accidental deaths (SMR = 138; p < .05). Among urban workers, however, there was a statistically significant two-fold excess compared to other Minnesotans. The greatest degree of excess occurred in 1975-1984 (SMR = 422; p < .05).

At greatest risk were workers who had been employed less than five years. Many of these deaths occurred away from on-the-job. Although present data do not permit a complete assessment, it was found that 14 out of 53 transportation deaths occurred at the workplace. There were 44 deaths from all other types of accidents, which was not greater than expected. Ten of these are known to have occurred on-the-job.

Significance

This study has shown that highway workers are at a substantially increased risk of dying from work related injuries. It also shows an increased risk of leukemia in long-term workers. Preliminary analyses have shown that both of these risks have persisted over the last decade. Based on these data the following actions are being implemented: 1) Highway maintenance worker mortlity will be updated in 5 and 10 years; 2) Case-control studies are being conducted to characterize any specific highway maintenance activities that may be associated with increased mortality risks; 3) A pilot study of injury surveillance is being designed; 4) Additional environmental monitoring for suspected exposures to hazardous agents will be conducted; and 5) A pilot study will be conducted using cytogentic assays to help assess personal and historical exposures to harmful substances.

Minimizing Dermal Exposure to Pesticides in Greenhouses

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Other Occupational Concerns
1 K01 OH00063-01
05/01/87 - 04/30/90
$32,270 ($32,270 Cum)

Summary

The major goal of this research is to test the effectiveness of a comprehensive exposure assessment program in reducing exposure among agricultural workers in greenhouses. Exposure will be evaluated simultaneously by air sampling, video imaging analysis of dermal deposition patterns produced by fluorescent tracer, and measurement of pesticide metabolites in urine. This evaluation will provide a basis for recommended changes in application procedures, worker hygiene, and protective clothing. Field investigations will include a survey of the critical variables influencing human exposure to pesticides in greenhouses, and comparative studies of exposure before and after control recommendations have been implemented. Within this experimental design, several technical issues will be addressed, including aerosol size distribution during applications, comparative behavior of the pesticide and tracer when sprayed, and environmental stability of the tracer compound. This research will lead to a clearer understanding of how pesticide exposure occurs, and how such exposure can be minimized through appropriate control strategies.

Hepatic Steatosis and Solvents: A Case-Control Study

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Other Occupational Concerns
1 K01 OH00071-01
09/29/87 - 09/28/89
$29,575 ($29,575 Cum)

Summary

The investigators propose to conduct a case-control study of the relationship of fatty liver disease to halogenated and unhalogenated hydrocarbon exposure. Cases will be obtained from five gastroenterology groups in Allegheny County. To ensure validity of results, two series of controls matched on age, gender, and race will be selected, one from the same gastroenterology referral source and one from the population registry of county. Exposures to halogenated and unhalogenated hydrocarbon exposures will be identified through occupational and environmental histories. Degree of exposure will be estimated in an ordinal fashion jointly by an industrial hygienist, an epidemiologist, and an occupational physician. Other known causes of steatosis including alcohol, obesity, diabetes, and hepatotoxic medications will be examined as effect modifiers.

Anemometry Related to Workplace Contaminant Distribution

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Other Occupational Concerns
5 R03 OH01825-02
05/01/84 - 04/30/87
$11,802 ($30,621 Cum)

Objectives

This research examines the relationship between air circulation in a test room and the spatial and temporal distributions of a tracer gas continuously released from a fixed point source. The aim is to develop empirical measures of ventilation, based on tracer gas techniques and anemometry, that can be used to estimate contaminant distributions and contaminant removal in ventilated spaces.

Methodology

This work uses a residence time model of room airflow to relate tracer gas measurements to local contaminant distributions and tracer dispersion. This approach characterizes flows by the local age distribution of fluid elements, which is directly related to the air concentrations in the room. The results provided by this technique are independent of the specific mixing mechanisms, simultaneously measuring the distribution of fresh air and the contaminant removal efficiency of the room ventilation system.

Air concentration distributions are studied in a 5100 ft^3 test chamber which has a controlled ventilation rate. A contaminant release is simulated by injecting a passive tracer gas at a point in the chamber The change in tracer concentrations with time is recorded by a computer controlled multi-port air sampler that continuously monitors air concentrations at up to 16 points in the room. Numerical integration of these data provides estimates of the local contaminant distributions and age distributions of the room air.

Local contaminant levels and age distributions are directly related to diffusive and bulk transport by turbulent air movements. Measurements of the air velocity vector can provide local estimates of both transport mechanisms, so that anemometry may give rapid, useful predictions of parameters for a residence time model.

Air movement is measured with a recently developed sonic anemometer that can accurately resolve the 3-dimensional wind vector at the low velocities found in a typical room. The velocity data provides estimates of the eddy diffusivity, the magnitude and direction of contaminant transport and predictions of local age distribution parameters. Computer mapping of the velocity data provides the general pattern of air movement and dispersal in the room. The values predicted from the anemometry will be compared to those measured with the tracer gas, at different ventilation conditions and source generation rates.

Progress and Accomplishments

We have completed modifications of the test chamber and air sampling system to allow tracer studies of air mixing. Tracer gas experiments indicate that a residence time model provides useful data on local ventilation rates and contaminant levels. The local mean age seems to be a temporally stable sure of ventilation at a point, for a given room geometry, temperature conditions, and room air flow rate.

A theoretical framework has been established for interpreting the air velocity data and relating these measurements to contaminant dispersal. A computerized fluid mechanics model of the room has been developed to estimate various length and velocity scale parameters. A numerical simulation which directly uses air velocity data to model dispersion from a point source is under development.

Significance

The ability to measure local differences in ventilation at various room locations has obvious applications for evaluating the effectiveness of room ventilation systems. Typing these measurements to local air concentrations provides a measuring index which is of primary interest to health scientists. The significance of this work arises from its longer-term potential to lead to a field deployable instrument and associated set of procedures for measuring and modeling contaminant dispersion in rooms, and determining how most efficiently to utilize or alter ventilation and source characteristics in a workplace to lower exposures.

Size Distribution of Airborne Lead Aerosol in Industry

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Other Occupational Concerns
1 R03 OH02044-02
11/01/84 - 01/31/87
$20,650 ($39,825 Cum)

Summary

The OSHA standard for control of exposure to inorganic lead is 50 $\mu g/m^3$ measured by total mass sampling. In setting the standard, OSHA assumed arbitrarily a constant value of 12.5 $\mu g/m^3$ as the amount of particulate 1 μm in size in any airborne sample, no matter what the source of total airborne concentration. This particle size distribution was then used to model the blood level distribution as a function of airborne lead concentration. The underlying hypothesis in this proposal is that the percentage of lead particulate less than 1μm in any sample is not fixed but varies according to the source of exposure, the aerosol generating process, and the resulting relationship between the percentage of small lead particulate and total airborne lead concentration. The particle size distribution is therefore determined by the source and conditions of exposure. If our hypothesis is borne out the OSHA PEL may be underprotective or overprotective depending on the source of lead aerosol generation.

The overall goal of the proposed research is to assess whether a lead PEL based on respirable sampling or respirable plus total mass sampling represents a more meaningful estimate of exposure. This would be accomplished by studying the impact of a variety of size distributions of lead in the predicted distribution of blood lead levels in workers exposed to airborne lead.

The specific objectives are:

1. Determine the particle size distribution of lead aerosol by cascade impactor in selected industrial processes selected to represent a range of distributions of particle size. This will be done using both stationary samples to characterize the process and personal samples to assess variability because of worker movement.
2. Model the distribution of blood lead values using each of the lead aerosol size distributions and the Bernard model for lead absorption, distribution, and excretion.
3. Characterize the magnitude of the differences in predicting blood lead distribution using the current OSHA total mass based method and a method which would differentiate respirable and non-respirable lead.

Size sampling will be carried out in a brass-bronze foundary, secondary smelter, and battery manufacturing plant using personal cascade impactors for both area and personal sampling. This research may enable the development of a lead exposure sampling scheme whose end result is better protection for lead exposed workers.

Publications

Hinds WC, Liu Wen-Chen V, Froines JR, Particle Bounce in a Personal Cascade Impactor: A Field Evaluation, Am Ind. Hyg. Assoc J. 46(9):517-523, 1985

SCBA Stressors: Effects of Carbon Dioxide Breathing

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Other Occupational Concerns
1 R03 OH02179-01A1
09/29/86 - 09/28/87
$18,476 ($18,476 Cum)

Summary

The primary stressors inherent in closed circuit self-contained breathing apparatuses (SCBAs) are: (1) resistance breathing; (2) carbon dioxide (CO_2) breathing; (3) hot air breathing; and (4) positive pressure breathing. Normally SCBAs keep CO_2 at a near 0% concentration, but as the CO_2 scrubber begins to fail the concentration rises exponentially. Presumably, if a high concentration can be tolerated for one hour, a smaller SCBA might be manufactured. This research was designed to (1) further refine a methodology for quantifying the effects of environmental stressors and neurotoxins on human cognition and subjective states, and (2) analyze data from a CO_2 experiment which provides useful information for recommending federal guidelines for the maximum allowable concentration of CO_2 in SCBAs.

The first objective - developing a stressor research methodology - was achieved in part by combining techniques developed by the National Research Council, Cambridge, UK, and the TNO Institute for Perception, Soesterberg, The Netherlands. Specifically, a serial choice reaction time (SCRT) task (see Leonard, 1959; Poulton, 1970; Wilkinson 1979) was made to test subjects according to the Additive Factors Method (AFM - see Sanders, 1980, 1983; Sternberg, 1969; Vercruyssen 1984). Another development was a means of monitoring perceived states via Subjective State Change Measures (SSCM) and clinical reactions via a forced-choice clinical symptoms inventory. The second objective - analysis of data from a CO_2 experiment - was achieved via multifactor repeated measures analysis of variance using Tukey WSD follow-up procedures where appropriate.

In short, this research provided a means of quantifying the effects of CO_2 on specific stages of information processing, as well as a clinical tool for monitoring subjective reactions. It determined that under the laboratory conditions employed breathing 4% CO_2 at rest causes a 20% impairment in the speed of processing information through the central nervous system and that the locus of this effect appears to be in the response selection stage. However, during exercise the effects of breathing 4% CO_2 disappear, presumably due to either a metabolic buffering of CO_2 in the system or a psychological mechanism like arousal or enhanced attentional state. Breathing 4% CO_2 for over one hour does not cause headaches at rest but does during exercise. In other words, the effects of breathing CO_2 manifest themselves in impaired cognition during rest and clinical symptoms (subjective discomfort) during exercise.

From an applied research perspective, these results are certainly useful for recommending revisions in the Federal Code 30 CFR, Part II. However, they are also of value in understanding the effects of breathing CO_2 in any situation where overexposures are a potential problem, e.g., space vehicles, submarines, agriculture silos, cargo hulls of ships. From a basic research perspective, this work found the AFM robust in quantifying stressor effects and in using serial rather than discrete choice reaction time as a criterion measure. Moreover, further evidence was gathered for the stochastic independence of information processing stages during serial choice reaction tasks.

Publications

Vercruyssen M, Yard S, White MK, Turner N, Mihaly T, Sever S: Quantifying Subjective Reactions to Wearing Respirators. Research Methodology. Abstract in proceedings and paper presented at the Int'l Society of Respiratory Protection Conference, Toronto, Canada, p 18-22, Oct 1987. Full manuscript in development

Vercruyssen M, Turner N, Mihaly T: SCBA Stressor Effects on Mental Abilities and Emotional States. Abstract in proceedings and paper presented at the International Society of Respiratory Protection Conference, Toronto, Canada, p 18-22 Oct 1987. Full Manuscript in development

Vercruyssen M, Turner N, Hodgson J, Kamon E: Behavioral Effects of Multiple SCBA Stressors. Safety Research for Recommending Federal Standards. Proc of the Human Factors Society 31st Annual Meeting, p 931-935, Santa Monica, California, 1987

Vercruyssen M, Sever S, Hancock PA: SCBA Research: Effects of Breathing Elevated Levels of CO_2 on CNS Function. Abstract submitted for presentation at the 1988 American Industrial Hygiene Association Conference, San Francisco, 1988

Measurement of Worker's Exposure to Styrene

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Other Occupational Concerns
1 R03 OH02180-01
05/01/86 - 04/30/88
$22,164 ($22,164 Cum)

Objectives

The research is investigating several factors which affect estimates of inhalation exposure to styrene which are made from personal monitor measurements. The initial objectives are to: 1) document the magnitude of the variability in the breathing zone concentrations of styrene within the reinforced plastics industry; and 2) study the relationship between exposure estimates made with personal samplers as they are commonly used and the inhalation exposure to styrene measured close to the nose. A third objective is to determine how the aerosols generated during the spraying process may contribute to the exposure. In addition, we will investigate the stability of samples of styrene collected on charcoal which are stored prior to analysis.

Methodology

Measurements are being made on workers in a boat manufacturing facility to compare the styrene concentration measured at commonly used personal sampler locations with the concentration measured at the nose. Additional information on sources and processes is being collected in order to understand some of the factors which contribute to the variability of the measurements. Additional studies carried out in front of a spraying booth will document the exposure of a spray operator that may result from the generated aerosols. Spray booth experiments are being carried out both in the field and in a laboratory testing facility. The stability of the styrene on charcoal is being studied by generating a uniform styrene atmosphere and collecting imultaneous samples, some of which are analyzed immediately and some stored for various time periods.

Progress and Accomplishments

A field study was conducted in a boat-manufacturing facility in Massachusetts. The production process involves spraying a mixture of styrene, catalysts and chopped fiberglass onto a shell which has been formed on a mold. A major purpose of the study was to document the magnitude of the variability in breathing zone concentrations of styrene during the spraying process. Concentration variations around individual workers were obvious, with the highest concentration found at the chest. The concentration measured by the nose sampler was about 76% of that measured by the chest sampler. A more detailed evaluation of the data is in progress. Also, the factors which contribute most significantly to those differences are being investigated.

To examine the effect of aerosols, airborne styrene levels were measured during four spraying experiments in an application testing laboratory. Two experiments involved the spraying of catalyzed polyester resin solution, which represents a typical process in the reinforced plastics industry. In the other two experiments, uncatalyzed polyester resin solution was sprayed in order to simulate a paint spraying process. Comparison of the samples collected with uncatalyzed and catalyzed resin permitted evaluation of the stability of the collected aerosols. The results of the four experiments showed that aerosols represented 30% ± 3% of the total styrene air concentration for the conditions used in this study. These results suggest that: 1) aerosol droplets represent a significant percentage of the total air concentration of styrene during resin spraying operations; 2) many of the particles generated during resin spraying operations are respirable; 3) the aerosol droplets may undergo physical and chemical changes such as vaporization and polymerization, which will affect the nature of respiratory exposure encountered and the dose received. Although the current study concentrated on the reinforced plastics industry where extensive exposure to styrene occurs, these results should be considered in other industrial situations involving spraying of volatile organic solvents. Exposure to volatile organic contaminants should not be assumed to be attributable to vapors only.

Significance

It is essential to know the reliability of the measurements used to estimate inhalation exposures in the reinforced plastic industry and to understand the factors which influence both the measurement and the true exposure. Worker exposure to volatile organic contaminants is evaluated by collecting breathing zone samples. This may not provide a true exposure estimate due to concentration variations within the breathing zone. Furthermore, exposure to volatile contaminants usually is attributed to vapors alone. This study demonstrated that aerosols, generated during the spraying of polyester resin solution, can contribute significantly to the exposure to volatile organic contaminants.

Finally, organic contaminants are usually collected by adsorption on a solid sorbent such as charcoal or silica gel. Sample stability prior to the analysis is essential in obtaining accurate exposure estimates. Many organic compounds are reactive and can undergo chemical reactions such as oxidation, hydrolysis, polymerization, etc. leading to underestimation to the true exposure of the species collected. The stability of styrene vapors on charcoal substrate has not yet been shown, particularly when peroxide, a polymerization initiator, is present.

Publications

Malek, RF, Daisey JM, Cohen BS: The Effect of Aerosol on Estimates of Inhalation Exposure to Airborne Styrene. Am Ind Hyg Assoc J 47: 524-529, 1986

Mechanisms of Cytoskeletal Injury by Heavy Metals

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Other Occupational Concerns
5 R03 OH02321-02
09/29/86 - 09/28/88
$20,068 ($50,905 Cum)

Objectives

The overall aim of the project is to investigate the possible mechanisms by which metal induced microtubule damage occurs in cultured 3T3 cells exposed to Cd^{+2}, Hg^{+2}, or As^{+3}. Because activated calmodulin is involved in regulating microtubule dynamics, the major hypothesis of the project is that Cd^{+2} and Hg^{+2} may affect microtubules by binding to and activating calmodulin. Because the ionic radii of Cd^{+2} (0.097) and Hg^{+2} (0.110) are so close to that of Ca^{+2} (0.099 nm), both ions are capable of substituting for Ca^{+2} in calmodulin. In contrast, As^{+3} (0.053 nm) is expected to affect microtubules via an interaction with tubulin sulfhydryls.

Methodology

The effects of metals on microtubule reassembly and on the microtubule networks in cultured cells and in detergent extracted cytoskeletons were monitored by indirect immunofluorescence microscopy and photography.

For the experiments involving *in vitro* microtubule assembly, bovine brain microtubule protein was purified according to the temperature-dependent disassembly-assembly method. Assembly of microtubules in vitro was done at 27°C and monitored spectrophotometrically by measuring the increase in turbidity at 350 nm following the addition of GTP to the reaction mixture. Permanent records of each experiment are provided by chart recordings of each turbidity scan.

The composition of bovine brain microtubule protein used for the *in vitro* assembly experiments was determined by discontinuous sodium dodecyl sulphate-polyacrylamide (7.5%) gel electrophoresis (SDS-PAGE), and staining with 0.25% Coomassie brilliant blue.

Progress and Accomplishments

Using the extracted cytoskeleton, we previously demonstrated that Cd^{+2}-induced microtubule disassembly can be prevented by the calmodulin inhibitors trifluoperazine or Compound 48/80. More recently, experiments were completed in which mixtures of Ca^{+2} and Cd^{+2}, at concentrations which by themselves do not affect microtubule networks, cause disassembly of microtubules in the extracted cytoskeleton. In addition, this Ca^{+2}/Cd^{+2}-induced microtubule disassembly can be prevented by Compound 48/80, further supporting the earlier conclusion that Cd^{+2} affects microtubules by activating calmodulin in a manner similar to Ca^{+2}. We also found that Hg^{+2} and CH_3Hg^+ are more potent inducers of microtubule disassembly in the extracted cytoskeleton than Cd^{+2}, and that calmodulin inhibitors had no effect on CH_3Hg^+-induced microtubule disassembly. However, inconsistent results with calmodulin inhibitors and Hg^{+2} were obtained.

The effects of metals on the kinetics of microtubule reassembly in 3T3 cells, following removal of the microtubule-disrupting drug colcemid were determined. Microtubule reassembly is inhibited by micromolar concentrations of Cd^{+2}, Hg^{+2}, As^{+3}, and CH_3Hg^+. This inhibition was originally planned to be monitored by measuring the lengths of fixed and immunofluorescently stained microtubules in photographs. However, ELISA protocols for more accurate quantitation of polymerized tubulin are being developed and standardized in this lab.

In order to more directly determine that Cd^{+2} can affect microtubules by binding to and activating calmodulin, we investigated the inhibitory effect of Cd^{+2} on the assembly of purified brain microtubule protein in vitro in the absence and presence of calmodulin. Micromolar concentrations of Cd^{+2} alone inhibited microtubule assembly. The addition of calmodulin enhanced the inhibitory effect of calmodulin, further reducing the rate. Furthermore, this enhanced inhibition is reversible by Compound 48/80. Calmodulin alone has no effect. The enhanced inhibition of microtubule assembly in the presence of Cd^{+2} and calmodulin, and its reversal by Compound 48/80, indicates that Cd^{+2} is binding to and activating calmodulin.

SDS-PAGE of microtubule protein used in the *in vitro* assembly experiments, and Coomassie blue staining of gels reveals the presence of tubulin and microtubule-associated protein (MAPs) in these preparations. Calmodulin has been shown to bind to MAPs in the presence of Ca^{+2}, and inhibit microtubule assembly, suggesting a possible mechanism for the Cd^{+2}/calmodulin-dependent enhancement of inhibition.

Significance

The results of this project indicate that microtubules vary in their sensitivity to the toxic metals Cd^{+2}, Hg^{+2}, As^{+3}, and CH_3Hg^{+}. In addition, the results demonstrate that Cd^{+2} can bind to and activate calmodulin, resulting in microtubule disassembly and inhibition of microtubule assembly *in vitro*. These findings raise the possibility that metal-induced microtubule damage may be involved in the expression of metal toxicity. More specifically, the finding that Cd^{+2} can substitute for Ca^{+2} in activating calmodulin, resulting in disturbances to microtubule dynamics, suggests that microtubule damage caused by the inappropriate activation of calmodulin by Cd^{+2} may be involved in Cd^{+2} toxicity.

Publications

Perrino BA, Chou IN: Inhibition of Microtubule Reassembly by Cadmium, Arsenite, and Methylmercury in Cultured Fibroblasts. The Toxicologist, 7: 71, 1987 (Abstract)

Perrino BA, Chou IN: Cytoskeletal Injury Resulting from the Interaction of Calmodulin with Metal Compounds. Proc of the Sixth Int'l Conference of Heavy Metals in the Environment, Vol. 1 p 332-336, 1987. This paper was presented as the Keynote Address of the Session on Health Effects: Cadmium and Mercury, Sept 16, 1987

Development of Methods to Estimate Beryllium Exposure

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Other Occupational Concerns
1 R03 OH02375-01
04/1/87 - 03/31/88
$21,222 ($21,222 Cum)

Summary

Beryllium exposure has been associated with the development of nonmalignant respiratory disease since the 1930s. More recently, the question of a causal association between exposure to beryllium and its compounds and the development of cancer has been raised. Several epidemiological studies of workers suggest an association between beryllium exposure and lung cancer but are limited by methodologic problems including use of duration of employment as a surrogate measure of exposure.

The National Institute for Occupational Safety and Health (NIOSH) has initiated a retrospective cohort mortality study of beryllium-exposed workers in order to resolve the methodologic challenges to the previous studies. Approximately 10,000 individuals fulfill the cohort definition at seven processing plants. A nested case-control study of lung cancer will be conducted to evaluate if exposure to beryllium is related to disease development. This epidemiological study will be substantially strengthened by the proposed research. The results of the estimated 6,000 environmental samples available at NIOSH and the Nuclear Regulatory Commission, formerly the Atomic Energy Commission, will be used to rank jobs by beryllium exposures. NIOSH does not plan to undertake the exposure assessment; however, the data are available to this investigator.

The specific aims of this proposal are:

1. To computerize all environmental data for the beryllium processing plants participating in the NIOSH study.
2. To rank exposures by work area and job title across all plants, and
3. To propose a strategy for reconstructing quantitative estimates of the exposures to beryllium.

The ranked exposure estimates will be available to NIOSH for an initial analysis of the case control study.

Formaldehyde Molecular States in the Gas Phase

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Other Occupational Concerns
7 R03 OH02377-01
09/28/84 - 05/31/87
$8,000 ($23,000 Cum)

Objectives

The research is to investigate the molecular species of formaldehyde present in the gas phase. Goals of the research are to: (1) validate an experimental protocol wherein formaldehyde vapors at equilibrium with formalin solution were found to consist of methylene glycol, methylal and the first three oligomers of polyoxymethylene glycol monomethyl ethers; and (2) determine the equilibrium constant for the formation of methylene glycol from formaldehyde and water in the vapor state.

Methodology

Validation of the gas phase trimethylsilylation method of analysis for formaldehyde condensate vapors has been conducted through vapor phase derivatization of chemically similar compounds. A mixture of N,O-bis (timethylsilyl) trifluoroacetamide (BSTFA) and N,N-dimethylformamide was placed in an evacuated vial and the vapors of alcohols, glycols and glycolic ethers were then introduced. Contents of the vial were analyzed by capillary gas chromatography with flame ionization detection. Yields were estimated through comparison of analytical results with the concentrations predicted from vapor pressure calculations.

The equilibrium constant for the formation of methylene glycol from formaldehyde and water in the gas phase will be determined over a range of temperatures of environmental and toxicological interest. Vapors of water and formaldehyde will be reacted in a dynamic dilution system and the effluents from the system analyzed by a matrix of analytical methods that collectively may distinguish monomeric formaldehyde, s-trioxane and the polyoxymethylene glycols.

Progress and Accomplishments

Validation of the gas phase derivatization of polar organics by BSTFA has been accomplished with the results listed in Table 1 below. The yields for most of the compounds studied were near quantitative and highly reproducible.

A monomeric formaldehyde generator has been constructed based on the acid catalyzed depolymerization of s-trioxane vapors. Gas chromatographic analysis of the generator effluent indicates complete conversion of s-trioxane to monomeric formaldehyde. Subsequent analysis of the effluent contents by the matrix of analytical methods for gas phase formaldehyde has yet to be performed. The gas phase reaction/dilution system for formaldehyde and water is being constructed.

TABLE 1. Yields for Gas Phase Trimethylsilylation of Polar Organics by BSTFA.

Compound	x	S	n
Methanol	87.5	2.9	3
Ethanol	91.8	3.3	4
Isopropanol	94.9	4.9	4
Phenol	68.4	1.1	3
Ethylene glycol	69.7	8.9	6
1,2-Propanediol	69.7	8.9	3
1,3-Butanediol	111.0	1.5	4
2-Methoxyethano	179.4	13.2	11
2-Ethoxyethanol	93.4	11.2	5

Significance

Gas phase trimethylsilylation of polar organics by BSTFA has essentially quantitative yields for many of the compounds analyzed. When viewed by chemical class, i.e. alcohols, glycols and glycolic ethers, the method averaged about eighty-five percent yield for each class with greater yields for the higher molecular weight compounds in each class. Results indicate that the method is good for approximation of the concentration of polar organics in the gas phase including polyoxymethylene glycols and polyoxymethylene glycol monomethyl ethers in formalin headspace. In addition, s-trioxane depolymerization under nitrogen gas is the recommended method for generation of monomeric formaldehyde since the absence of water in this system prevents the formation of formaldehyde oligomers.

Quantifying Professional Judgment in Industrial Hygiene

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Other Occupational Concerns
1 R03 OH02553-01
09/29/87 - 09/28/88
$18,101 ($18,101 Cum)

Objectives

This pilot study explores subjective exposure estimation by industrial hygienists and will attempt to determine how well on the average a group of industrial hygienists can estimate occupational exposures they have not themselves sampled.

Methodology

Worker exposures are being measured extensively using passive monitors in a batch chemical processing facility. Results of this exposure assessment will constitute the known distribution of exposures. Additionally, data about process conditions, ventilation, and worker tasks will be collected.

A random sample of 25 industrial hygienists with experience measuring occupational exposures in batch chemical processing areas will be generated and asked to participate in personal interviews. Each personal interview will follow a formal interview protocol (set of questions) in the decision analytic tradition. The interview will include a review of possible cognitive biases affecting subjective estimation of exposures, a review of the processes, conditions, and worker tasks in the exposure scenario, and an elicitation of subjective exposure estimates for the workers in the process area.

A statistical analysis will determine how well on average the 25 industrial hygienists can predict the median exposure and the body and tails of the exposure distribution.

Progress and Accomplishments

To date, exposure data collection is in progress and industrial hygienist selection methods have been developed.

Significance

Industrial hygienists are frequently asked to subjectively estimate exposures both prospectively and also retrospectively for use in occupational epidemiological studies. The results of this pilot study will be a first look at the ability of industrial hygienists to perform these tasks, providing some insight into how accurate and reliable such subjective exposure estimates actually are.

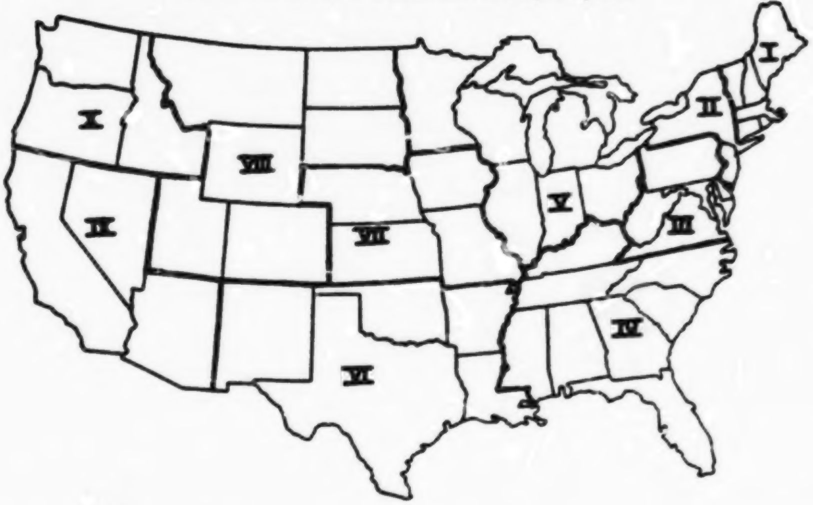
GRANTS ACTIVE DURING FY87

	COMPETING GRANTS		TOTAL GRANTS	
	No. of Awards	Amount of Awards	No. of Awards	Amount of Awards
Grants from FY87 Budget ($6.501M)				
Research Project Grants (R01)	21	$2,460,370	48	$5,735,581
Career Development Grants (K01)	5	$159,034	9	$291,073
Small Grants (R03)	11	$243,345	15	$333,768
Other Grants	1	$50,000	2	$140,578
Subtotal	38	$2,912,749	74	$6,501,000
Grants from FY86 Budget ($6.221M)				
Research Project Grants (R01)	2	$228,961	15	$1,712,399
Career Development Grants (K01)	0	$0	9	$281,743
Small Grants (R03)	7	$153,678	13	$256,042
Other Grants	2	$58,629	3	$145,143
Subtotal	11	$441,268	40	$2,395,327
Grants from FY85 Budget ($6.50M)				
Research Project Grants (R01)	0	$0	6	$591,956
Career Development Grants (K01)	0	$0	0	$0
Small Grants (R03)	0	$0	3	$24,882
Other Grants	0	$0	0	$0
Subtotal	0	$0	9	$616,838
Awards from all Years				
Research Project Grants (R01)	23	$2,689,331	69	$8,039,936
Career Development Grants (K01)	5	$159,034	18	$572,816
Small Grants (R03)	18	$397,023	31	$614,692
Other Grants	3	$108,629	5	$285,721
TOTAL	49	$3,354,017	123	$9,513,165

FY87 GRANT AWARDS BY PROGRAM AREA

Program Area	Competing Grants No. of Awards	 Amount of Awards	Total Grants No. of Awards	 Amount of Awards	 Amt. Per.
Occupational Lung Diseases	4	$215,737	10	$842,127	13%
Musculoskeletal Injuries	1	$23,866	2	$200,611	3%
Occupational Cancers	2	$79,000	6	$313,762	5%
Traumatic Injuries	2	$243,952	2	$243,952	4%
Cardiovascular Diseases	0	$0	0	$0	0%
Disorders of Reproduction	4	$88,501	9	$433,215	6%
Neurotoxic Disorders	0	$0	6	$859,792	13%
Noise-Induced Hearing Loss	2	$229,307	4	$521,895	8%
Dermatologic Disorders	2	$123,120	2	$123,120	2%
Psychologic Disorders	1	$118,781	3	$379,557	6%
Engineering Control Systems	4	$202,814	5	$293,489	5%
Respirator Research	4	$467,899	5	$517,153	8%
Other Occupational Concerns	12	$1,119,772	20	$1,772,327	27%
TOTAL	38	$2,912,749	74	$6,501,000	100%

FY87 GRANT AWARDS BY REGION AND STATE
74 GRANTS TOTALLING $6,501,000



	No.	Amt.	(Per.)
Region I	9	$729,327	(11.2%)
Connecticut	1	$160,896	(2.5%)
Massachusetts	7	$442,987	(6.8%)
New Hampshire	0	$0	(0.0%)
Rhode Island	1	$125,444	(1.9%)
Vermont	0	$0	(0.0%)
Region III	9	$758,358	(11.7%)
Maryland	2	$214,954	(3.3%)
Pennsylvania	6	$414,729	(6.4%)
Virginia	1	$127,964	(2.0%)
*West Virginia	0	$711	(0.0%)
Region V	19	$1,510,506	(23.2%)
Illinois	1	$92,802	(1.4%)
Indiana	2	$184,247	(2.8%)
Michigan	3	$322,151	(5.0%)
Minnesota	2	$96,109	(1.5%)
Ohio	10	$638,452	(9.8%)
Wisconsin	1	$176,745	(2.7%)
Region VII	3	$311,828	(4.8%)
Iowa	1	$70,038	(1.1%)
Kansas	1	$112,642	(1.7%)
Missouri	1	$129,148	(2.0%)
Region IX	10	$949,364	(14.6%)
Arizona	3	$234,848	(3.6%)
California	7	$714,516	(11.0%)

	No.	Amt.	(Per.)
Region II	8	$937,667	(14.4%)
New Jersey	1	$32,270	(0.5%)
New York	7	$905,397	(13.9%)
Region IV	6	$710,264	(10.9%)
Alabama	0	$0	(0.0%)
Florida	0	$0	(0.0%)
Georgia	0	$0	(0.0%)
Kentucky	0	$0	(0.0%)
North Carolina	5	$583,404	(9.0%)
Tennessee	1	$126,860	(1.9%)
Region VI	8	$531,556	(8.2%)
Arkansas	2	$139,814	(2.2%)
Louisiana	2	$64,026	(1.0%)
Oklahoma	1	$123,518	(1.9%)
Texas	3	$204,198	(3.1%)
Region VIII	0	$1,728	(0.0%)
*Colorado	0	$1,728	(0.0%)
Utah	0	$0	(0.0%)
Region X	2	$54,287	(0.8%)
Oregon	0	$0	(0.0%)
Washington	2	$54,287	(0.8%)
Foreign	0	$7,015	(0.1%)
*Finland	0	$7,015	(0.1%)

***Received supplemental funding. Not counted as an award in FY87.**

GRANT NUMBER INDEX BY GRANT ACTIVITY

Research Project Grants (R01)

Grant Number	Principal Investigator	Current Funding	Year	Page
5 R01 OH 00823-08	Abou-Donia, Mohamed B.,Ph.D.	$157,703	87	98
5 R01 OH 00835-09	Swartz, William J., Ph.D.	$41,526	87	74
5 R01 OH 00923-05	Yu, C.P., Ph.D.	$58,351	85	10
5 R01 OH 00952-08	Scheving, Lawrence E., Ph.D.	$119,699	87	101
2 R01 OH 00978-07	Trudell, James R., Ph.D.	$267,434	98	170
5 R01 OH 01035-04	Graham, William G., M.D.	$287,892	86	13
5 R01 OH 01122-06	Kauffman, Charles, W., Ph.D.	$99,184	86	70
7 R01 OH 01152-08	Henderson, Donald, Ph.D.	$163,440	87	116
2 R01 OH 01301-04A1	Willeke, Klaus, Ph.D.	$183,006	87	154
2 R01 OH 01331-04A1	Bakale, George, Ph.D.	$160,935	86	55
5 R01 OH 01485-03	Liu, Benjamin, Ph.D.	$98,842	85	155
5 R01 OH 01520-03	Thysen, Benjamin, Ph.D.	$190,839	86	76
5 R01 OH 01595-03	Hinds, William C., Sc.D.	$62,011	85	157
5 R01 OH 01603-03	Nichols, Larry D., Ph.D.	$131,948	85	159
2 R01 OH 01630-05	Uyeki, Edwin M., Ph.D.	$112,642	87	172
1 R01 OH 01632-01A2	Zimmerman, Neil J., Ph.D.	$134,247	87	160
5 R01 OH 01644-03	Underhill, Dwight W., Sc.D.	$76,656	86	161
5 R01 OH 01646-03	Corn, Morton, Ph.D.	$119,866	85	163
5 R01 OH 01910-03	Hemminki, Kari J., Ph.D.	$81,657	86	78
1 R01 OH 01929-01A1	Cook, Thomas M., M.S.	$68,026	86	48
5 R01 OH 01932-03	Perkins, Jimmy L., Ph.D.	$72,733	86	143
5 R01 OH 01968-03	Barnett, Rosalind C., Ph.D.	$228,376	87	136
5 R01 OH 01970-03	Hammad, Yehia Y., Sc.D.	$41,644	86	15
5 R01 OH 01972-03	De Caprio, Anthony P., Ph.D.	$46,290	86	104
1 R18 OH 01981-01A3	Clark, C. Scott, Ph.D.	$156,344	87	175
5 R01 OH 02003-03	Abou-Donia, Mohamed B., Ph.D.	$198,511	87	106
2 R01 OH 02005-04	Harber, Philip I., M.D.	$118,246	87	165
5 R01 OH 02020-03	Sickles, Dale W., Ph.D.	$64,881	86	108
5 R01 OH 02021-03	Stedman, Donald, Ph.D.	$87,649	86	127
5 R01 OH 02024-03	Drury, Colin G., Ph.D.	$108,627	86	138
5 R01 OH 02027-03	Corbett, Michael, Ph.D.	$73,722	86	57
5 R01 OH 02029-04	Donham, Kelley J., D.V.M.	$70,038	87	17
5 R01 OH 02065-03	Spencer, Peter S., Ph.D.	$191,861	87	110
5 R01 OH 02066-02	Levine, Steven P., Ph.D.	$120,938	85	177
5 R01 OH 02067-03	Swanson, G. Marie, Ph.D.	$258,679	86	59
5 R01 OH 02076-03	Carter, Dean E., Ph.D.	$131,079	87	19
5 R01 OH 02084-03	Walsh, Patrick R., M.D.	$176,745	87	49
2 R01 OH 02091-03	Rheins, Lawrence A., Ph.D.	$96,630	87	128
5 R01 OH 02104-02	Asal, Nabih R., Ph.D.	$123,518	87	61
5 R01 OH 02108-02	Pellizzari, Edo D., Ph.D.	$148,195	87	179
5 R01 OH 02114-02	Merrill, William W., M.D.	$160,896	87	21
5 R01 OH 02116-03	Johnson, Christopher M., M.D.	$63,709	87	23
5 R01 OH 02122-03	Fischbein, Alf, M.D.	$50,323	87	63
5 R01 OH 02128-04	Clark, William W., Ph.D.	$129,148	87	118
5 R01 OH 02132-03	Esmen, Nurtan A., Ph.D.	$90,675	87	145
5 R01 OH 02148-03	Cleary, Stephen F., Ph.D.	$127,964	87	80

GRANT NUMBER INDEX BY GRANT ACTIVITY

Grant Number	Principal Investigator	Current Funding	Year	Page
5 R01 OH 02149-02	Ansari, Ghulam A.S., Ph.D.	$92,030	87	181
5 R01 OH 02154-02	Evans, John S., Sc.D.	$49,254	87	166
1 R01 OH 02162-01A1	Mansfield, Phyllis K., Ph.D.	$118,781	87	140
5 R01 OH 02185-02	Aposhian, H. Vasken, Ph.D.	$71,369	87	25
5 R01 OH 02191-03	Boekelheide, Kim, M.D.	$125,444	87	83
1 R01 OH 02214-01A1	Brown, William E., Ph.D.	$131,054	87	183
5 R01 OH 02221-02	Rappaport, Stephen, M., Ph.D.	$135,524	87	185
1 R01 OH 02241-01A1	Rossignol, Annette M., Sc.D.	$61,398	87	72
1 R01 OH 02254-01A1	Stewart, Walter F., Ph.D.	$182,554	87	73
5 R01 OH 02264-02	Kennedy, Thomas P., M.D.	$126,860	87	27
1 R01 OH 02284-01	Glazer, Eva R., M.D.	$19,540	87	64
1 R01 OH 02288-01	Harber, Philip I., M.D.	$106,294	87	187
5 R01 OH 02303-02	Levine, Steven P., Ph.D.	$95,360	87	188
2 R01 OH 02317-03	Hamernik, Roger P., Ph.D.	$179,307	87	120
1 R01 OH 02329-01	Garrison, Richard P., Ph.D.	$102,904	87	147
1 R01 OH 02332-01	Bergofsky, Edward H., M.D.	$128,448	87	28
5 R01 OH 02351-02	Draper, William M., Ph.D.	$39,331	87	65
7 R01 OH 02384-01	Letz, Richard E., Ph.D.	$159,618	87	112
1 R01 OH 02392-01	Flynn, Michael R., Sc.D.	$57,245	87	149
1 R01 OH 02404-01	Levine, Steven P., Ph.D.	$123,887	87	189
1 R01 OH 02413-01	Rice, Carol H., Ph.D.	$59,460	87	67
1 R01 OH 02417-01	Hjelle, Joseph T., Ph.D.	$92,802	87	190
1 R01 OH 02586-01	Leigh, J. Paul, Ph.D.	$28,147	87	191

Career Development Grants (K01)

5 K01 OH 00002-03	Reiser, Karen M., M.D.	$32,400	86	29
5 K01 OH 00007-03	Shatos, Marie A., Ph.D.	$32,400	86	31
5 K01 OH 00017-03	Guy, Richard H., Ph.D.	$31,174	86	130
5 K01 OH 00018-03	Kreiss, Kathleen, M.D.	$34,128	86	32
5 K01 OH 00019-03	Lantz, Robert C., Ph.D.	$27,235	86	34
5 K01 OH 00022-03	Cohen, Beverly S., Ph.D.	$32,400	86	36
5 K01 OH 00028-03	White, Roberta F., Ph.D.	$32,400	87	141
5 K01 OH 00035-03	Prichard, Howard M., Ph.D.	$30,563	86	85
5 K01 OH 00042-03	Talbott, Evelyn O., Ph.D.	$32,400	86	123
5 K01 OH 00054-02	Costa, Lucio, G., Ph.D.	$32,400	87	192
5 K01 OH 00055-02	Parker, David L., M.D.	$32,400	87	194
5 K01 OH 00059-02	Caruso, Rita L., Ph.D.	$31,482	86	87
1 K01 OH 00063-01	Fenske, Richard A., Ph.D.	$32,270	87	196
7 K01 OH 00064-03	Letz, Richard E., Ph.D.	$32,400	87	113
1 K01 OH 00065-01	Wey, Howard E., Ph.D.	$32,389	87	38
1 K01 OH 00067-01	Hanna, Linda M., Ph.D.	$32,400	87	39
1 K01 OH 00068-01	Crutchfield, Clifton D., Ph.D.	$32,400	87	167
1 K01 OH 00071-01	Hodgson, Michael J., M.D.	$29,575	87	197

GRANT NUMBER INDEX BY GRANT ACTIVITY

Grant Number	Principal Investigator	Current Funding	Year	Page
	Small Grants (R03)			
5 R03 OH 01825-02	Spear, Robert C., Ph.D.	$11,802	85	198
5 R03 OH 01827-02	Magee, Carol A., M.P.H.	$5,080	85	88
5 R03 OH 01990-02	Kowal, Charles D., M.D.	$10,642	86	133
5 R03 OH 02044-02	Froines, John R., Ph.D.	$20,650	86	200
5 R03 OH 02095-02	Dusenbery, David B., Ph.D.	$14,748	86	115
5 R03 OH 02098-02	Guernsey, Judith R., Ph.D.	$20,723	86	40
5 R03 OH 02101-02	Flynn, Michael R., Sc.D.	$14,175	86	150
5 R03 OH 02141-02	Feth, Lawrence L., Ph.D.	$21,426	86	125
1 R03 OH 02178-01A1	Pytel, Jean L., Ph.D.	$23,866	87	51
1 R03 OH 02179-01A1	Vercruyssen, Max, Ph.D.	$18,476	86	201
1 R03 OH 02180-01	Cohen, Beverly S., Ph.D.	$22,164	86	203
1 R03 OH 02189-01	Ashizawa, Eiko A., M.P.H.	$24,675	86	42
1 R03 OH 02229-01	Yates, James W., Ph.D.	$20,693	86	52
1 R03 OH 02236-01A1	Murphy, Dennis J., Ph.D.	$20,778	87	151
7 R03 OH 02238-02	Talaska, Glenn G., Ph.D.	$21,206	86	68
5 R03 OH 02243-02	Connor, Thomas H., Ph.D.	$21,590	87	69
5 R03 OH 02258-02	Gandy, Jay, Ph.D.	$20,115	87	90
1 R03 OH 02265-01	Witek, Theodore J., Dr.P.H.	$23,000	86	44
1 R03 OH 02287-01	Chung, Min K., Ph.D.	$23,464	86	54
5 R03 OH 02321-02	Perrino, Brian A.	$26,068	87	205
5 R03 OH 02345-02	Sublet, Virginia H., Ph.D.	$22,650	87	91
1 R03 OH 02375-01	Rice, Carol H., Ph.D.	$21,222	87	207
1 R03 OH 02376-01	Hamilton, John D., M.Sc.	$22,650	87	93
7 R03 OH 02377-01	Utterback, David F., Ph.D.	$8,000	85	208
1 R03 OH 02380-01	Hueston, William D., Ph.D.	$21,177	87	95
1 R03 OH 02383-01	Shortridge, Linda A.	$22,924	87	96
1 R03 OH 02422-01	Daniell, William, M.D.	$21,887	87	152
1 R03 OH 02425-01	O'Neil, Carol E., Ph.D.	$22,500	87	46
1 R03 OH 02433-01	Leung, Mun-Fai	$26,490	87	135
1 R03 OH 02548-01	Savitz, David A., Ph.D.	$21,750	87	97
1 R03 OH 02553-01	Evans, John S., Sc.D.	$18,101	87	210
	Conference Grants (R13)			
1 R13 OH 02117-01	Lockey, James E., M.D.	$10,000	86	47
	Small Business Grants (R43, R44)			
5 R44 OH 01951-03	Bruce, Robert D.	$86,514	86	153
1 R43 OH 02312-01	Stetter, Joseph R., Ph.D.	$48,629	86	168
1 R43 OH 02313-01A1	Cameron, Hugh R.	$50,000	87	126

PRINCIPAL INVESTIGATOR INDEX BY GRANT ACTIVITY

Research Project Grants (R01)

Principal Investigator	Area	Grant Number	Page
Abou-Donia, Mohamed B., Ph.D.	Neuro	5 R01 OH 00823-08	98
Abou-Donia, Mohamed B., Ph.D.	Neuro	5 R01 OH 02003-03	106
Ansari, Ghulam A.S., Ph.D.	Other	5 R01 OH 02149-02	181
Aposhian, H. Vasken, Ph.D.	Lung	5 R01 OH 02185-02	25
Asal, Nabih R., Ph.D.	Cancer	5 R01 OH 02104-02	61
Bakale, George, Ph.D.	Cancer	2 R01 OH 01331-04A1	55
Barnett, Rosalind C., Ph.D.	Psycho	5 R01 OH 01968-03	136
Bergofsky, Edward H., M.D.	Lung	1 R01 OH 02332-01	28
Boekelheide, Kim, M.D.	Repro	5 R01 OH 02191-03	83
Brown, William E., Ph.D.	Other	1 R01 OH 02214-01A1	183
Carter, Dean E., Ph.D.	Lung	5 R01 OH 02076-03	19
Clark, C. Scott, Ph.D.	Other	1 R18 OH 01981-01A3	175
Clark, William W., Ph.D.	Noise	5 R01 OH 02128-04	118
Cleary, Stephen F., Ph.D.	Repro	5 R01 OH 02148-03	80
Cook, Thomas M., M.S.	Muscu	1 R01 OH 01929-01A1	48
Corbett, Michael, Ph.D.	Cancer	5 R01 OH 02027-03	57
Corn, Morton, Ph.D.	Respir	5 R01 OH 01646-03	163
De Caprio, Anthony P., Ph.D.	Neuro	5 R01 OH 01972-03	104
Donham, Kelley J., D.V.M.	Lung	5 R01 OH 02029-04	17
Draper, William M., Ph.D.	Cancer	5 R01 OH 02351-02	65
Drury, Colin G., Ph.D.	Psycho	5 R01 OH 02024-03	138
Esmen, Nurtan A., Ph.D.	Contr	5 R01 OH 02132-03	145
Evans, John S., Sc.D.	Respir	5 R01 OH 02154-02	166
Fischbein, Alf, M.D.	Cancer	5 R01 OH 02122-03	63
Flynn, Michael R., Sc.D.	Contr	1 R01 OH 02392-01	149
Garrison, Richard P., Ph.D.	Contr	1 R01 OH 02329-01	147
Glazer, Eva R., M.D.	Cancer	1 R01 OH 02284-01	64
Graham, William G., M.D.	Lung	5 R01 OH 01035-04	13
Hamernik, Roger P., Ph.D.	Noise	2 R01 OH 02317-03	120
Hammad, Yehia Y., Sc.D.	Lung	5 R01 OH 01970-03	15
Harber, Philip I., M.D.	Respir	2 R01 OH 02005-04	165
Harber, Philip I., M.D.	Other	1 R01 OH 02288-01	187
Hemminki, Kari J., Ph.D.	Repro	5 R01 OH 01910-03	78
Henderson, Donald, Ph.D.	Noise	7 R01 OH 01152-08	116
Hinds, William C., Sc.D.	Respir	5 R01 OH 01595-03	157
Hjelle, Joseph T., Ph.D.	Other	1 R01 OH 02417-01	190
Johnson, Christopher M., M.D.	Lung	5 R01 OH 02116-03	23
Kauffman, Charles, W., Ph.D.	Trauma	5 R01 OH 01122-06	70
Kennedy, Thomas P., M.D.	Lung	5 R01 OH 02264-02	27
Leigh, J. Paul, Ph.D.	Other	1 R01 OH 02586-01	191
Letz, Richard E., Ph.D.	Neuro	7 R01 OH 02384-01	112
Levine, Steven P., Ph.D.	Other	5 R01 OH 02066-02	177
Levine, Steven P., Ph.D.	Other	5 R01 OH 02303-02	188
Levine, Steven P., Ph.D.	Other	1 R01 OH 02404-01	189
Liu, Benjamin, Ph.D.	Respir	5 R01 OH 01485-03	155
Mansfield, Phyllis K., Ph.D.	Psycho	1 R01 OH 02162-01A1	140
Merrill, William W., M.D.	Lung	5 R01 OH 02114-02	21

PRINCIPAL INVESTIGATOR INDEX BY GRANT ACTIVITY

Principal Investigator	Area	Grant Number	Page
Nichols, Larry D., Ph.D.	Respir	5 R01 OH 01603-03	159
Pellizzari, Edo D., Ph.D.	Other	5 R01 OH 02108-02	179
Perkins, Jimmy L., Ph.D.	Contr	5 R01 OH 01932-03	143
Rappaport, Stephen, M., Ph.D.	Other	5 R01 OH 02221-02	185
Rheins, Lawrence A., Ph.D.	Derma	2 R01 OH 02091-03	128
Rice, Carol H., Ph.D.	Cancer	1 R01 OH 02413-01	67
Rossignol, Annette M., Sc.D.	Trauma	1 R01 OH 02241-01A1	72
Scheving, Lawrence E., Ph.D.	Neuro	5 R01 OH 00952-08	101
Sickles, Dale W., Ph.D.	Neuro	5 R01 OH 02020-03	108
Spencer, Peter S., Ph.D.	Neuro	5 R01 OH 02065-03	110
Stedman, Donald, Ph.D.	Derma	5 R01 OH 02021-03	127
Stewart, Walter F., Ph.D.	Trauma	1 R01 OH 02254-01A1	73
Swanson, G. Marie, Ph.D.	Cancer	5 R01 OH 02067-03	59
Swartz, William J., Ph.D.	Repro	5 R01 OH 00835-09	74
Thysen, Benjamin, Ph.D.	Repro	5 R01 OH 01520-03	76
Trudell, James R., Ph.D.	Other	2 R01 OH 00978-07	170
Underhill, Dwight W., Sc.D.	Respir	5 R01 OH 01644-03	161
Uyeki, Edwin M., Ph.D.	Other	2 R01 OH 01630-05	172
Walsh, Patrick R., M.D.	Muscu	5 R01 OH 02084-03	49
Willeke, Klaus, Ph.D.	Respir	2 R01 OH 01301-04A1	154
Yu, C.P., Ph.D.	Lung	5 R01 OH 00923-05	10
Zimmerman, Neil J., Ph.D.	Respir	1 R01 OH 01632-01A2	160

Career Development Grants (K01)

Caruso, Rita L., Ph.D.	Repro	5 K01 OH 00059-02	87
Cohen, Beverly S., Ph.D.	Lung	5 K01 OH 00022-03	36
Costa, Lucio, G., Ph.D.	Other	5 K01 OH 00054-02	192
Crutchfield, Clifton D., Ph.D.	Respir	1 K01 OH 00068-01	167
Fenske, Richard A., Ph.D.	Other	1 K01 OH 00063-01	196
Guy, Richard H., Ph.D.	Derma	5 K01 OH 00017-03	130
Hanna, Linda M., Ph.D.	Lung	1 K01 OH 00067-01	39
Hodgson, Michael J., M.D.	Other	1 K01 OH 00071-01	197
Kreiss, Kathleen, M.D.	Lung	5 K01 OH 00018-03	32
Lantz, Robert C., Ph.D.	Lung	5 K01 OH 00019-03	34
Letz, Richard E., Ph.D.	Neuro	7 K01 OH 00064-03	113
Parker, David L., M.D.	Other	5 K01 OH 00055-02	194
Prichard, Howard M., Ph.D.	Repro	5 K01 OH 00035-03	85
Reiser, Karen M., M.D.	Lung	5 K01 OH 00002-03	29
Shatos, Marie A., Ph.D.	Lung	5 K01 OH 00007-03	31
Talbott, Evelyn O., Ph.D.	Noise	5 K01 OH 00042-03	123
Wey, Howard E., Ph.D.	Lung	1 K01 OH 00065-01	38
White, Roberta F., Ph.D.	Psycho	5 K01 OH 00028-03	141

Small Grants (R03)

Ashizawa, Eiko A., M.P.H.	Lung	1 R03 OH 02189-01	42
Chung, Min K., Ph.D.	Muscu	1 R03 OH 02287-01	54
Cohen, Beverly S., Ph.D.	Other	1 R03 OH 02180-01	203
Connor, Thomas H., Ph.D.	Cancer	5 R03 OH 02243-02	69

PRINCIPAL INVESTIGATOR INDEX BY GRANT ACTIVITY

Principal Investigator	Area	Grant Number	Page
Daniell, William, M.D.	Contr	1 R03 OH 02422-01	152
Dusenbery, David B., Ph.D.	Neuro	5 R03 OH 02095-02	115
Evans, John S., Sc.D.	Other	1 R03 OH 02553-01	210
Feth, Lawrence L., Ph.D.	Noise	5 R03 OH 02141-02	125
Flynn, Michael R., Sc.D.	Contr	5 R03 OH 02101-02	150
Froines, John R., Ph.D.	Other	5 R03 OH 02044-02	200
Gandy, Jay, Ph.D.	Repro	5 R03 OH 02258-02	90
Guernsey, Judith R., Ph.D.	Lung	5 R03 OH 02098-02	40
Hamilton, John D., M.Sc.	Repro	1 R03 OH 02376-01	93
Hueston, William D., Ph.D.	Repro	1 R03 OH 02380-01	95
Kowal, Charles D., M.D.	Derma	5 R03 OH 01990-02	133
Leung, Mun-Fai	Derma	1 R03 OH 02433-01	135
Magee, Carol A., M.P.H.	Repro	5 R03 OH 01827-02	88
Murphy, Dennis J., Ph.D.	Contr	1 R03 OH 02236-01A1	151
O'Neil, Carol E., Ph.D.	Lung	1 R03 OH 02425-01	46
Perrino, Brian A.	Other	5 R03 OH 02321-02	205
Pytel, Jean L., Ph.D.	Muscu	1 R03 OH 02178-01A1	51
Rice, Carol H., Ph.D.	Other	1 R03 OH 02375-01	207
Savitz, David A., Ph.D.	Repro	1 R03 OH 02548-01	97
Shortridge, Linda A.	Repro	1 R03 OH 02383-01	96
Spear, Robert C., Ph.D.	Other	5 R03 OH 01825-02	198
Sublet, Virginia H., Ph.D.	Repro	5 R03 OH 02345-02	91
Talaske, Glenn G., Ph.D.	Cancer	7 R03 OH 02238-02	68
Utterback, David F., Ph.D.	Other	7 R03 OH 02377-01	208
Vercruyssen, Max, Ph.D.	Other	1 R03 OH 02179-01A1	201
Witek, Theodore J., Dr.P.H.	Lung	1 R03 OH 02265-01	44
Yates, James W., Ph.D.	Muscu	1 R03 OH 02229-01	52

Conference Grants (R13)

Principal Investigator	Area	Grant Number	Page
Lockey, James E., M.D.	Lung	1 R13 OH 02117-01	47

Small Business Grants (R43, R44)

Principal Investigator	Area	Grant Number	Page
Bruce, Robert D.	Contr	5 R44 OH 01951-03	153
Stetter, Joseph R., Ph.D.	Respir	1 R43 OH 02312-01	168
Cameron, Hugh R.	Noise	1 R43 OH 02313-01A1	126

GRANTEE INSTITUTION INDEX BY GRANT ACTIVITY

Research Project Grants (R01)

Grantee Institution	Principal Investigator	Page
Brown University	Boekelheide, Kim, M.D.	83
California Public Health Foundation	Draper, William M., Ph.D.	65
Carnegia-Mellon University	Brown, William E., Ph.D.	183
Case Western Reserve University	Bakale, George, Ph.D.	55
Central Institute for the Deaf	Clark, William W., Ph.D.	118
Duke University	Abou-Donia, Mohamed B., Ph.D.	106
Duke University	Abou-Donia, Mohamed B., Ph.D.	98
Harvard School of Public Health	Evans, John S., Sc.D.	166
Institute of Occupational Health, Finland	Hemminki, Kari J., Ph.D.	78
Johns Hopkins University	Corn, Morton, Ph.D.	163
Johns Hopkins University	Stewart, Walter F., Ph.D.	73
Louisiana State University Medical Center	Swartz, William J., Ph.D.	74
Mayo Foundation	Johnson, Christopher M., M.D.	23
Medical College of Georgia	Sickles, Dale W., Ph.D.	108
Medical College of Wisconsin	Walsh, Patrick R., M.D.	49
Michigan Cancer Foundation	Swanson, G. Marie, Ph.D.	59
Moleculon Research Corporation	Nichols, Larry D., Ph.D.	159
Mount Sinai School of Medicine	Fischbein, Alf, M.D.	63
Mount Sinai School of Medicine	Letz, Richard E., Ph.D.	112
New York State Department of Health	De Caprio, Anthony P., Ph.D.	104
Northern California Cancer Program	Glazer, Eva R., M.D.	64
Pennsylvania State University	Mansfield, Phyllis K., Ph.D.	140
Purdue University	Zimmerman, Neil J., Ph.D.	160
Research Foundation of SUNY	Hamernik, Roger P., Ph.D.	120
Research Triangle Institute	Pellizzari, Edo D., Ph.D.	179
San Jose State University	Leigh, J. Paul, Ph.D.	191
Stanford University	Trudell, James R., Ph.D.	170
State University of New York	Henderson, Donald, Ph.D.	116
State University of New York	Yu, C.P., Ph.D.	10
State University of New York	Drury, Colin G., Ph.D.	138
State University of New York Research Foundation	Bergofsky, Edward H., M.D.	28
Tufts University	Rossignol, Annette M., Sc.D.	72
Tulane University	Hammad, Yehia Y., Sc.D.	15
University of Alabama, Birmingham	Perkins, Jimmy L., Ph.D.	143
University of Arizona	Aposhian, H. Vasken, Ph.D.	25
University of Arizona	Carter, Dean E., Ph.D.	19
University of Arkansas	Scheving, Lawrence E., Ph.D.	101
University of California, Berkeley	Rappaport, Stephen, M., Ph.D.	185
University of California, Los Angeles	Harber, Philip I., M.D.	165
University of California, Los Angeles	Harber, Philip I., M.D.	187
University of California, Los Angeles	Hinds, William C., Sc.D.	157
University of Cincinnati	Willeke, Klaus, Ph.D.	154
University of Cincinnati	Rheins, Lawrence A., Ph.D.	128
University of Cincinnati	Clark, C. Scott, Ph.D.	175
University of Cincinnati	Rice, Carol H., Ph.D.	67
University of Denver	Stedman, Donald, Ph.D.	127
University of Florida	Corbett, Michael, Ph.D.	57

GRANTEE INSTITUTION INDEX BY GRANT ACTIVITY

Grantee Institution	Principal Investigator	Page
University of Illinois, Peoria	Hjelle, Joseph T., Ph.D.	190
University of Iowa	Cook, Thomas M., M.S.	48
University of Iowa	Donham, Kelley J., D.V.M.	17
University of Kansas Medical Center	Uyeki, Edwin M., Ph.D.	172
University of Michigan, Ann Arbor	Levine, Steven P., Ph.D.	189
University of Michigan, Ann Arbor	Garrison, Richard P., Ph.D.	147
University of Michigan, Ann Arbor	Levine, Steven P., Ph.D.	177
University of Michigan, Ann Arbor	Levine, Steven P., Ph.D.	188
University of Michigan, Ann Arbor	Kauffman, Charles, W., Ph.D.	70
University of Minnesota	Liu, Benjamin, Ph.D.	155
University of North Carolina	Flynn, Michael R., Sc.D.	149
University of Oklahoma	Asal, Nabih R., Ph.D.	61
University of Pittsburgh	Esmen, Nurtan A., Ph.D.	145
University of Pittsburgh	Underhill, Dwight W., Sc.D.	161
University of Tennessee	Kennedy, Thomas P., M.D.	27
University of Texas Medical Branch	Ansari, Ghulam A.S., Ph.D.	181
University of Vermont	Graham, William G., M.D.	13
Virginia Commonwealth University	Cleary, Stephen F., Ph.D.	80
Wellesley College	Barnett, Rosalind C., Ph.D.	136
Yale University School of Medicine	Merrill, William W., M.D.	21
Yeshiva University	Spencer, Peter S., Ph.D.	110
Yeshiva University	Thysen, Benjamin, Ph.D.	76

Career Development Grants (K01)

Grantee Institution	Principal Investigator	Page
Boston University	White, Roberta F., Ph.D.	141
Johns Hopkins University	Hanna, Linda M., Ph.D.	39
Minnesota Department of Health	Parker, David L., M.D.	194
Mount Sinai School of Medicine	Letz, Richard E., Ph.D.	113
National Jewish Center	Kreiss, Kathleen, M.D.	32
New York University Medical Center	Cohen, Beverly S., Ph.D.	36
Rutgers University	Fenske, Richard A., Ph.D.	196
University of Arizona	Crutchfield, Clifton D., Ph.D.	167
University of California, Davis	Reiser, Karen M., M.D.	29
University of California, San Francisco	Guy, Richard H., Ph.D.	130
University of Cincinnati	Wey, Howard E., Ph.D.	38
University of Michigan, Ann Arbor	Caruso, Rita L., Ph.D.	87
University of Pittsburgh	Hodgson, Michael J., M.D.	197
University of Pittsburgh	Talbott, Evelyn O., Ph.D.	123
University of Texas	Prichard, Howard M., Ph.D.	85
University of Vermont	Shatos, Marie A., Ph.D.	31
University of Washington	Costa, Lucio, G., Ph.D.	191
West Virginia University	Lantz, Robert C., Ph.D.	34

Small Grants (R03)

Grantee Institution	Principal Investigator	Page
Boston University	Leung, Mun-Fai	135
Boston University	Perrino, Brian A.	205
Georgia Institute of Technology	Dusenbery, David B., Ph.D.	115
Harvard School of Public Health	Evans, John S., Sc.D.	210

GRANTEE INSTITUTION INDEX BY GRANT ACTIVITY

Grantee Institution	Principal Investigator	Page
Harvard School of Public Health	Flynn, Michael R., Sc.D.	150
Johns Hopkins University	Magee, Carol A., M.P.H.	88
Johns Hopkins University	Ashizawa, Eiko A., M.P.H.	42
Mount Sinai School of Medicine	Witek, Theodore J., Dr.P.H.	44
New York University Medical Center	Cohen, Beverly S., Ph.D.	203
Ohio State University Research Foundation	Hueston, William D., Ph.D.	95
Pennsylvania State University	Murphy, Dennis J., Ph.D.	151
Pennsylvania State University	Pytel, Jean L., Ph.D.	51
Tulane Medical School	O'Neil, Carol E., Ph.D.	46
University of Arkansas	Gandy, Jay, Ph.D.	90
University of Arkansas	Talaska, Glenn G., Ph.D.	68
University of California, Berkeley	Spear, Robert C., Ph.D.	198
University of California, Los Angeles	Froines, John R., Ph.D.	200
University of Cincinnati	Hamilton, John D., M.Sc.	93
University of Cincinnati	Rice, Carol H., Ph.D.	207
University of Cincinnati	Shortridge, Linda A.	96
University of Cincinnati	Sublet, Virginia H., Ph.D.	91
University of Illinois	Chung, Min K., Ph.D.	54
University of Iowa	Guernsey, Judith R., Ph.D.	40
University of Kansas	Feth, Lawrence L., Ph.D.	125
University of Louisville	Yates, James W., Ph.D.	52
University of North Carolina	Savitz, David A., Ph.D.	97
University of Pittsburgh	Kowal, Charles D., M.D.	133
University of South Carolina	Utterback, David F., Ph.D.	208
University of Southern California	Vercruyssen, Max, Ph.D.	201
University of Texas Health Science Center	Connor, Thomas H., Ph.D.	69
University of Washington	Daniell, William, M.D.	152

Conference Grants (R13)

University of Utah	Lockey, James E., M.D.	47

Small Business Grants (R43, R44)

Camtech, Inc.	Cameron, Hugh R.	126
Hoover Keith & Bruce Inc	Bruce, Robert D.	153
Transducer Research, Inc.	Stetter, Joseph R., Ph.D.	168

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